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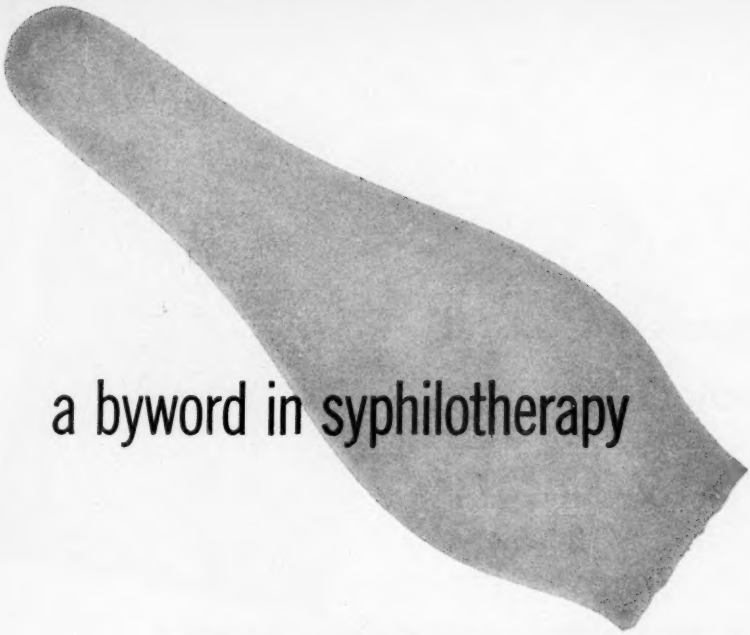

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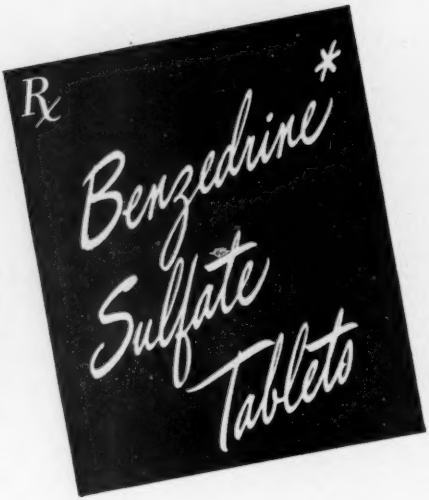

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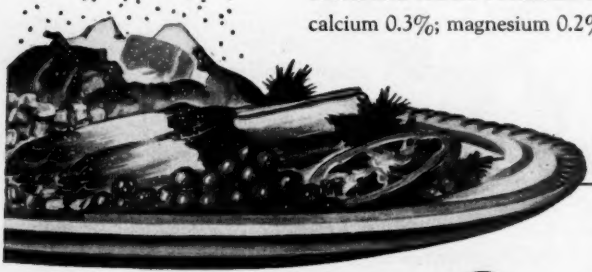

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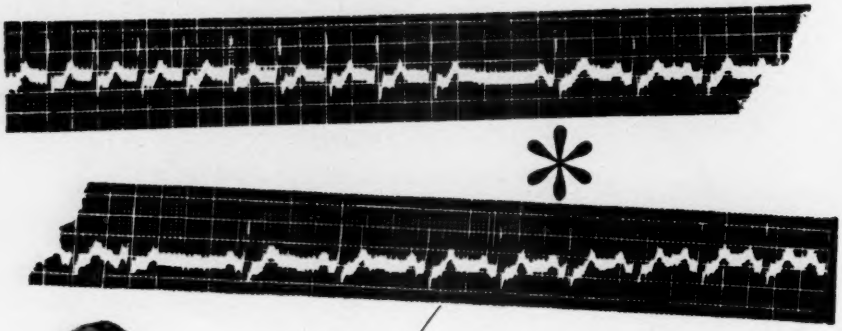

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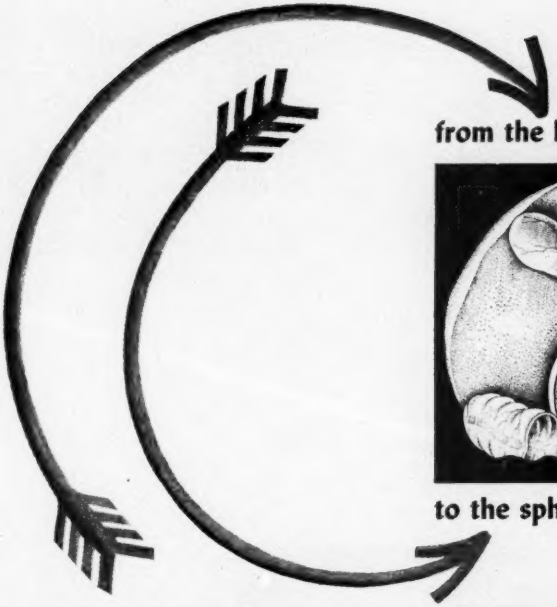

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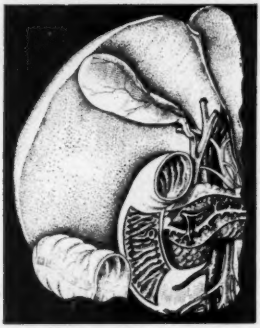

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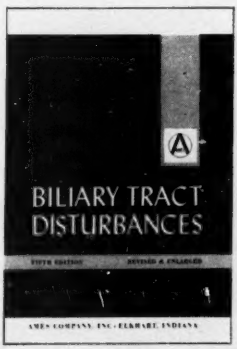

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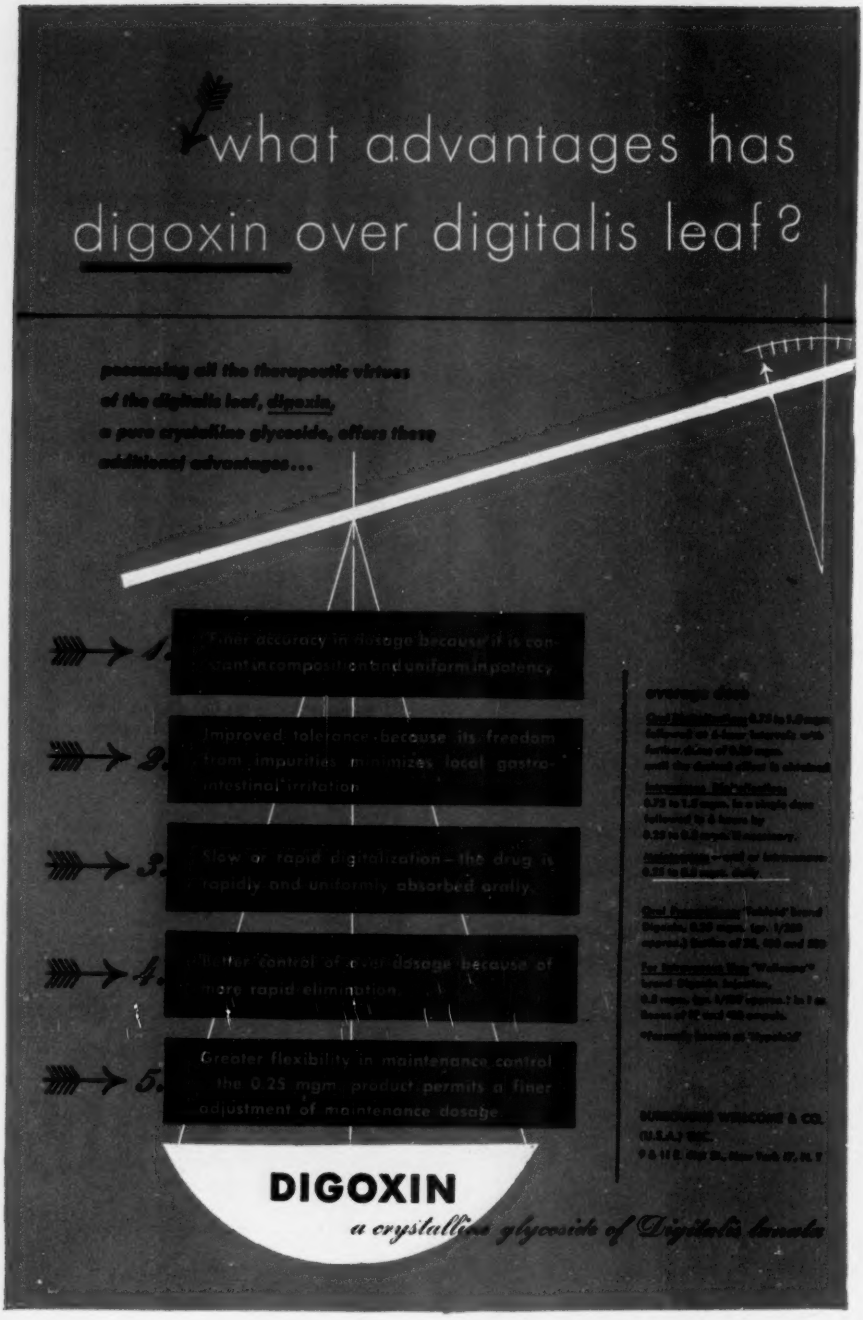
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VOLUME 85 FEBRUARY 1950 NUMBER 2

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OBSERVATIONS ON THE PHYSIOLOGIC EFFECTS OF CORTISONE AND ACTH IN MAN

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UNTIL recently inadequate supplies of the adrenal cortex hormone 17-hydroxy-11-dehydrocorticosterone [1] (formerly known as "compound E" and hereinafter called "cortisone") and pituitary adrenocorticotropic hormone (hereinafter called "ACTH") have limited studies of the effects of these substances in man when administered in amounts sufficient to have pronounced physiologic activity.

With the development and improvement of methods for partial synthesis of cortisone and for the separation of larger amounts of ACTH from the pituitary glands of animals, adequate amounts of both materials became available for trial in certain diseases of human beings. A great impetus was given to their production by recent observations concerning their favorable influence on the course of a common chronic disease (rheumatoid arthritis) as well as in a common acute disease

From the Division of Medicine (Drs. Sprague, Hench, Slocumb and Polley), the Division of Biochemistry (Drs. Power, Mason and Kendall); the Endocrinology Laboratory, Section on Clinical Physiology (Dr. Albert), and the Division of Clinical Laboratories (Dr. Mathieson), Mayo Clinic and Foundation.

1. Compound E was first isolated from beef adrenal glands and described by Mason, H. L.; Myers, C. S., and Kendall, E. C.: The Chemistry of Crystalline Substances Isolated From the Suprarenal Gland, J. Biol. Chem. **114**:613 (July) 1936. It was also described in that year by Reichstein, T.: Ueber Bestandteile der Nebennierenrinde: VI. Trennungsmethoden, sowie Isolierung der Substanzen Fa., H und J., Helvet. chim. acta **19**:1107, 1936, and by Wintersteiner, O. and Pfiffner, J. J.: Chemical Studies on the Adrenal Cortex: III. Isolation of Two New Physiologically Inactive Compounds, J. Biol. Chem. **116**:291 (Nov.) 1936.

with serious chronic sequelae (acute rheumatic fever).[2] In the short time since these observations were made, increasing amounts of these hormones have become available and, although they are still scarce, various investigators have learned much concerning their effects in man.

It is important that the effects of these materials on man be made known to the medical profession because such information is of interest from a purely investigative standpoint and because it bears on the possible therapeutic use of these hormones in the future should sufficient quantities become generally available.

The purpose of this paper is to present a brief review of the effects of these hormones on animals and man prior to the present era of their clinical application and to present a review of our observations of their physiologic effects in man since September 1948. Special attention will not be given to questions of practical therapeutics. However, it is realized that such questions are implicit in any discussion of these hormones. Our experience with cortisone has been far more extensive than that with ACTH; therefore, cortisone will receive the greater attention. There is, however, no definite line of demarcation between the physiologic effects of these two agents. The results obtained through the use of cortisone apply in most respects to those with ACTH with the single exception of dosage: A quantitative measure of the amount of secretion delivered by the adrenal cortex after stimulation by ACTH is not possible.

PREVIOUS KNOWLEDGE OF PHYSIOLOGIC EFFECTS OF CORTISONE AND ACTH

Studies of Animals.—Cortisone: The physiologic effects of cortisone and allied adrenal hormones on animals can be briefly summarized as follows[3]: They (*a*) maintain life of adrenalectomized animals; (*b*) increase resistance of adrenalectomized animals to various forms of stress; (*c*) affect protein and carbohydrate metabolism and induce hyperglycemia, glycosuria and negative nitrogen balance; (*d*) destroy white blood cells (lymphocytes and eosinophils) and lymph nodes; (*e*) affect metabolism of electrolytes; (*f*) produce atrophy and depression of function of the adrenal cortices and atrophy of the thymus

2. (*a*) Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: The Effect of a Hormone of the Adrenal Cortex (17-Hydroxy-11-Dehydrocorticosterone: Compound E) and of Pituitary Adrenocorticotropic Hormone on Rheumatoid Arthritis, Proc. Staff Meet., Mayo Clin. **24**:181 (April 13) 1949. (*b*) Hench, P. S.; Slocumb, C. H.; Barnes, A. R.; Smith, H. L.; Polley, H. F., and Kendall, E. C.: The Effects of the Adrenal Cortical Hormone 17-Hydroxy-11-Dehydrocorticosterone (Compound E) on the Acute Phase of Rheumatic Fever: Preliminary Report, ibid. **24**:277 (May 25) 1949.

3. Since so many investigators have contributed to this field of knowledge, detailed references will not be given.

gland; (*g*) increase capacity for muscle work in adrenalectomized animals (this effect is not observed in animals with intact adrenal glands), and (*h*) increase antihyaluronidase activity.

ACTH: The major physiologic effects of ACTH are contingent on stimulation of the adrenal cortices to produce steroid hormones, some of them closely allied to cortisone. Thus, ACTH, through the medium of adrenal cortex hormones, influences protein and carbohydrate metabolism as cited in the last paragraph (*c*), has effects on white blood cells as cited in (*d*), alters the metabolism of electrolytes, induces atrophy of the thymus and produces increases in antihyaluronidase activity.

Studies of Human Subjects.—Previous knowledge of the effects of cortisone and ACTH on man has come directly from observations following the administration of small amounts of these materials, principally to normal subjects and to subjects having Addison's disease, and indirectly from studies of the various manifestations of Cushing's syndrome. Many of the features of the latter condition may be regarded as being due to overproduction of cortisone-like steroid hormones by hyperfunctioning adrenal cortices; hence patients with this syndrome present a unique opportunity for the study of the consequences of chronic excess of such hormones.

Cortisone: The carbohydrate activity of cortisone is readily demonstrable in subjects with Addison's disease.[4] Its administration abolishes the tendency of some patients with Addison's disease to become hypoglycemic when food is withheld, decreases sensitivity to insulin, increases the threshold at which symptoms of hypoglycemia appear and may increase slightly the level of the fasting blood sugar. Nitrogen excretion in the urine may be increased. Carbohydrate activity is

4. (*a*) Thorn, G. W.; Koepf, G. F.; Lewis, R. A., and Olsen, E. F.: Carbohydrate Metabolism in Addison's Disease, J. Clin. Investigation **19**:813 (Nov.) 1940. (*b*) Sprague, R. G.; Gastineau, C. F.; Mason, H. L., and Power, M. H.: Effects of Synthetic 11-Dehydrocorticosterone (Compound A) in a Subject with Addison's Disease, Am. J. Med. **4**:175 (Feb.) 1948. (*c*) Sprague, R. G.; Power, M. H., and Mason, H. L.: Studies of the Effects of Adrenal Cortical Hormones on Carbohydrate Metabolism in Human Subjects, Proc. Am. Diabetes A., to be published. (*d*) Thorn, G. W., and Forsham, P. H.: Metabolic Changes in Man Following Adrenal and Pituitary Hormone Administration: Recent Advances in Hormone Research, to be published. (*e*) Sprague, R. G.; Power, M. H.; Mason, H. L., and Cluxton, H. E.: Metabolic Effects of Synthetic Compound E (17-Hydroxy-11-Dehydrocorticosterone) in Two Patients with Addison's Disease and in One with Coexisting Addison's Disease and Diabetes Mellitus, abstracted, J. Clin. Investigation, **28**:812 (July) 1949. (*f*) Thorn, G. W., and others: Clinical and Metabolic Changes in Addison's Disease Following Administration of Compound E Acetate (11-Dehydro, 17-Hydroxy-Corticosterone Acetate), cited by Thorn and others.[9]

even more strikingly demonstrated in subjects who have coexisting Addison's disease and diabetes mellitus.[5] Administration of cortisone to them results in a pronounced increase in the excretion of glucose, nitrogen and ketone bodies and an increase in the requirement for insulin. An intensification of glycosuria and increased requirement for insulin also has been observed in diabetic patients without Addison's disease.[6] In 1 such patient with coexisting rheumatoid arthritis the daily requirement for insulin increased from a pretreatment level of 10 units to 50 units daily.[6b]

The electrolyte effects of cortisone in man need further study. Early investigators, employing single small doses of the hormone in the dog and rat, suggested that it and related hormones might cause an increased excretion of sodium and chloride.[7] Later studies on human subjects with Addison's disease have indicated that it may cause retention of salt and water.[8] In any event, it is apparent that its effects on electrolyte metabolism are of much less magnitude than those of desoxycorticosterone acetate.

A slight depressor effect of cortisone on the resting blood pressure of hypertensive patients has been reported.[6a]

Although experience is still meager, evidence is accumulating that cortisone is a valuable addition to the therapy of Addison's disease. Evidence to date suggests that relatively small doses of the hormone (10 to 20 mg. daily) are required for adequate clinical control of chronic adrenal cortex insufficiency.[9] Whether or not by itself it will

5. (*a*) Sprague, R. G.; Kepler, E. J.; Keating, F. R., Jr., and Power, M. H.: Coexisting Addison's Disease and Diabetes Mellitus: Comparative Effects of Compound E (17-Hydroxy-11-Dehydrocorticosterone) and Allied Substances in Three Cases, abstracted, J. Clin. Investigation **26**:1198 (Nov.) 1947. (*b*) Balfour, W. M., and Sprague, R. G.: Association of Diabetes Mellitus and Disorders of the Anterior Pituitary, Thyroid and Adrenal Cortex, Am. J. Med. **7**:596 (Nov.) 1949.

6. (*a*) Perera, G. A.; Pines, K. L.; Hamilton, H. B., and Vislocky, K.: Clinical and Metabolic Study of 11-Dehydro-17-Hydroxy-Corticosterone Acetate (Kendall Compound E) in Hypertension, Addison's Disease and Diabetes Mellitus, Am. J. Med. **7**:56 (July) 1949. (*b*) Boland, E. W., and Headley, N. E.: Effects of Cortisone Acetate on Rheumatoid Arthritis, J. A. M. A. **141**:301 (Oct. 1) 1949.

7. Thorn, G. W.; Engel, L. L., and Lewis, R. A.: The Effect of 17-Hydroxycorticosterone and Related Adrenal Cortical Steroids on Sodium and Chloride Excretion, Science **94**:348 (Oct. 10) 1941.

8. (*a*) Perera, Pines, Hamilton and Vislocky.[6a] (*b*) Sprague, Gastineau, Mason and Power.[4b]

9. Thorn, G. W.; Bayles, T. B.; Massell, B. F.; Forsham, P. H.; Hill, S. R., Jr.; Smith, S., III; and Warren, J. E.: Studies on the Relation of Pituitary-Adrenal Function to Rheumatic Disease, New England J. Med. **241**:529 (Oct. 6) 1949.

be adequate replacement therapy for prolonged periods or whether the concurrent use of desoxycorticosterone acetate will be necessary to control electrolyte metabolism remains to be demonstrated. In some patients with Addison's disease abnormal electroencephalographic tracings have reverted to normal during administration of cortisone.[4d] During administration of the hormone to 2 female patients with Addison's disease it was observed that small fractions of the amount given were excreted in the urine as corticosteroids (reducing steroids) and as 17-ketosteroids.[4e]

ACTH: Reference will be made only to pertinent points of a few published studies of ACTH. There is evidence that the hormone is capable of stimulating all known functions of the adrenal cortices of man.[10] The degree of stimulation has been variable, depending doubtless on the amount and potency of the ACTH employed, the nature of the subject and other factors. The excretion of 17-ketosteroids and corticosteroids in the urine increases[10]; diabetogenic effects have been demonstrated occasionally, and excretion of uric acid and total nitrogen is increased.[11] The lysis of eosinophils which follows stimulation of the adrenals by ACTH has become the basis for a useful test for the demonstration of the functional capacity of the glands.[12] Evidence also suggests that lysis of lymphoid tissue and an increase in the neutrophils of the peripheral blood occur under the influence of ACTH.[13] Effects on metabolism of electrolytes have been demonstrated. Androgenic effects have been described.[10a]

Even prior to the time that cortisone and ACTH were administered in relatively large amounts to human subjects, many of their physiologic effects might have been predicted with approximate accuracy by examining the features of Cushing's syndrome. As already suggested, many of the manifestations of this syndrome are explainable on the basis of overproduction of cortisone-like steroid hormones by

10. (*a*) Mason, H. L.; Power, M. H.; Rynearson, E. H.; Ciaramelli, L. C.; Li, C. H., and Evans, H. M.: Results of Administration of Anterior Pituitary Adrenocorticotropic Hormone to a Normal Human Subject, J. Clin. Endocrinol. **8**:1 (Jan.) 1948. (*b*) Forsham, P. H.; Thorn, G. W.; Prunty, F. T. G., and Hills, A. G.: Clinical Studies with Pituitary Adrenocorticotrophin, ibid. **8**:15 (Jan.) 1948.

11. Conn. J. W.; Louis, L. H., and Wheeler, C. E.: Production of Temporary Diabetes Mellitus in Man with Pituitary Adrenocorticotropic Hormone: Relation to Uric Acid Metabolism, J. Lab. & Clin. Med. **33**:651 (June) 1948.

12. Thorn, G. W.; Forsham, P. H.; Prunty, F. T. G., and Hills, A. G.: A Test for Adrenal Cortical Insufficiency; Response to Pituitary Adrenocorticotropic Hormone, J. A. M. A. **137**:1005 (July 17) 1948.

13. Hills, A. G.; Forsham, P. H., and Finch, C. A.: Changes in Circulating Leukocytes Induced by the Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man, Blood **3**:755 (July) 1948.

hyperplastic or neoplastic adrenal cortices.[14] Among the features of the syndrome which might be attributed to excessive amounts of this type of hormonal material are impairment of carbohydrate tolerance, weakness and wasting of muscle, osteoporosis, thinning and striation of the skin, tendency to bruise easily, lymphopenia, eosinopenia and an increased excretion of corticosteroids in the urine. Furthermore, in cases of Cushing's syndrome associated with a tumor of one adrenal cortex, the commonly found atrophy of the contralateral adrenal cortex could readily be a consequence of overproduction of cortisone-like material by the adrenal neoplasm.

Thus, in spite of the limited quantities of cortisone and ACTH which have been available in the past, there is, nevertheless, a considerable background of information, derived more from study of animals than from study of human beings, to serve as a basis for further investigation of the effects of these hormones in man.

MATERIAL AND METHODS

Patients Studied.—The data to be reported were derived from a study of 33 patients who received cortisone, cortisone acetate[15] or ACTH.[16] In addition, 1 of the patients received 17-hydroxycorticosterone (hereinafter referred to as "compound F").[17] Cortisone and compound F were administered intramuscularly, in one or two doses daily, as saline suspensions of finely ground crystals containing 25 mg. per cubic centimeter. ACTH was administered intramuscularly

14. (*a*) Albright, F.: Cushing's Syndrome: Its Pathological Physiology, Its Relationship to the Adreno-Genital Syndrome, and Its Connection with the Problem of the Reaction of the Body to Injurious Agents ("Alarm Reaction" of Selye), in Harvey Lectures, 1942-1943, Baltimore, Williams & Wilkins Company, 1943, p. 123. (*b*) Kepler, E. J.; Sprague, R. G.; Mason, H. L., and Power, M. H.: The Pathologic Physiology of Adrenal Cortical Tumors and Cushing's Syndrome, in Pincus, G.: Recent Progress in Hormone Research: The Proceedings of the Laurentian Hormone Conference (1947-1948), New York, Academic Press, Inc., 1948, vol. 2, p. 345.

15. Inasmuch as there seem to be no important differences in the physiologic effects of cortisone and cortisone acetate, both compounds will be referred to henceforth as cortisone. It is realized that 100 mg. of cortisone acetate is presumably equivalent physiologically to 89 mg. of the free compound and that there may be minor differences in the timing of the effect of the two compounds owing to different solubilities. Merck & Co., Inc. (Dr. James M. Carlisle, Medical Director) supplied the cortisone used in this study.

16. Armour and Company (Dr. John R. Mote, Director of Medical Research) supplied the ACTH. The oxytocin content of the different preparations of ACTH varied from less than 0.4 unit to 0.48 units per vial. Each vial contained the equivalent of 25 mg. of Armour standard ACTH LA-1-A. The pressor content was 2 units per vial.

17. The Upjohn Company and Dr. E. Gifford Upjohn supplied the 17-hydroxycorticosterone (compound F) which was used in the study of this patient.

TABLE 1.—*Clinical Data and Hormones Administered in Thirty-Three Cases Studied*

Case*	Age, Yr.	Sex	Diagnosis	Hormones Administered: Substance	Days	Total Amount Gm.	Average Daily Dose, Mg.
1	29	F	Rheumatoid arthritis	Cortisone	130	11.125	86
				Cortisone acetate	57	5.95	104
2	41	M	Rheumatoid arthritis and spondylitis	Cortisone	75	7.5	100
				Cortisone†	21	2.1	100
				Cortisone acetate	119	12.0	101
3	64	M	Rheumatoid arthritis	Cortisone	60	6.0	100
				Cortisone†	16	1.6	100
				Cortisone acetate	69	6.9	100
				Cortisone acetate†	73	7.3	100
4	41	M	Rheumatoid arthritis	Cortisone	12	1.2	100
				Cortisone†	27	2.575	95
				Cortisone acetate	12	1.3	108
				Cortisone acetate†	71	6.6	93
5	34	M	Rheumatoid arthritis and spondylitis	Cortisone	67	4.95	74
				Cortisone acetate	66	6.175	94
6	49	F	Rheumatoid arthritis	Cortisone	10	1.0	100
				ACTH†	12	1.2	100
				Compound F†	12	0.9	75
				Cortisone acetate†	30	4.2	140
7	53	F	Rheumatoid arthritis	Cortisone	25	2.5	100
8	62	M	Rheumatoid arthritis	Cortisone acetate	9	1.1	122
				Cortisone acetate†	18	3.6	200
9	44	F	Rheumatoid arthritis	Cortisone acetate	25	2.15	86
10	49	M	Rheumatoid arthritis	Cortisone acetate	61	9.25	152
				Cortisone acetate†	40	6.1	153
11	40	F	Rheumatoid arthritis	Cortisone acetate	8	1.0	125
				ACTH†	12	1.26	105
				Cortisone acetate†	18	3.6	200
12	43	F	Rheumatoid arthritis	Cortisone acetate	57	5.9	104
				Cortisone acetate†	57	5.7	100
13	31	F	Rheumatoid arthritis	Cortisone acetate	29	3.7	128
14	45	F	Rheumatoid arthritis	ACTH	12	1.2	100
				Cortisone acetate†	30	4.2	140
				Cortisone acetate†	18	3.6	200
				Cortisone acetate†	18	3.6	200
15	29	M	Rheumatoid arthritis	Cortisone acetate	64	8.25	129
				ACTH†	31	2.975	96
16	49	M	Rheumatoid arthritis	Cortisone acetate	161	18.85	117
17	23	M	Rheumatoid arthritis and spondylitis	ACTH	11	0.77	70
18	38	M	Rheumatoid arthritis and spondylitis	ACTH	87	9.665	111
19	30	M	Rheumatoid spondylitis	Cortisone acetate	66	6.8	103
20	55	F	Rheumatoid arthritis	Cortisone acetate	22	2.4	109
21	30	F	Rheumatoid arthritis	Cortisone acetate	56	5.6	100
22	22	M	Rheumatoid arthritis and spondylitis	Cortisone acetate	32	3.2	100
23	17	F	Rheumatoid arthritis	Cortisone acetate	38	4.0	105
				Cortisone acetate†	20	2.0	100
24	15	F	Acute rheumatic fever	Cortisone acetate	38	4.65	122
25	17	M	Acute rheumatic fever	Cortisone acetate	24	4.3	179
				Cortisone acetate†	28	3.1	111
26	16	M	Acute rheumatic fever	Cortisone acetate	22	3.45	157
27	14	F	Acute rheumatic fever	Cortisone acetate	40	5.2	130
28	14	M	Acute rheumatic fever	ACTH	23	0.386	17
29	13	F	Acute rheumatic fever	ACTH	38	0.825	22
30	14	M	Acute rheumatic fever	ACTH	16	0.53	33
31	18	M	Acute rheumatic fever	ACTH	24	0.675	28
				ACTH	23	0.760	33
32	32	F	Disseminated lupus erythematosus	Cortisone acetate	166	17.775	107
33	25	F	Psoriasis, psoriatic arthritis	Cortisone acetate	43	4.5	105
				ACTH	28	3.6	129

* Cases 1 through 16 are the same cases as those bearing these numbers in the report of Hench, Kendall, Slocumb and Polley.[2a] Cases 24, 25 and 26 are the same as cases 1, 8 and 2 respectively, in the report of Hench, Slocumb, Barnes, Smith, Polley and Kendall.[2b]

† New course of administration.

in saline solutions in three or four doses daily. Solutions were freshly prepared before each dose. The clinical and metabolic changes which are to be described are principally those associated with prolonged (in contrast to short term) administration of the hormones. As anticipated, there was, in general, a direct relation between the daily dose and duration of administration of the hormones, on the one hand, and the magnitude of the physiologic effects on the other. Seventeen of the patients had rheumatoid arthritis; 5 had rheumatoid arthritis and spondylitis; 2 had rheumatoid spondylitis; 8 had acute rheumatic fever; 1 had disseminated lupus erythematosus, and 1, psoriasis and psoriatic arthritis. The scheme of numbering of cases, as well as the data on age and sex and the hormones administered, are given in table 1.

Method of Study.—The patients were under careful clinical observation each day. In most cases, determinations of 17-ketosteroids and corticosteroids in urine and chemical and hematologic studies of blood were made at intervals. In some cases glucose tolerance tests were performed; in some, the response of the adrenal cortices to a single dose of 25 mg. of ACTH, as estimated by changes in the number of circulating eosinophils,[12] was tested before, during and after administration of cortisone.

Seven patients with rheumatoid arthritis (5 women and 2 men) volunteered to be the subjects of metabolic balance studies for potassium, sodium, chloride, nitrogen, calcium and phosphorus. The subjects of such studies were maintained in a special metabolic unit of the hospital. Activity was kept approximately uniform from day to day, with some inevitable variations consequent to exacerbations and remissions of the rheumatoid arthritis. The food given during these studies was weighed. Three daily menus were employed in rotation in the study of each patient; each menu was used twice in every six day period.[18] Intake of water (as drinking water) was kept constant (usually 1,500 cc.) from day to day. Urine was collected by periods of twenty-four hours. Chemical analyses of urine were performed daily or on the pooled specimens of two or three days. Feces were collected and analyzed by periods of six days; a carmine marker was utilized for their separation by successive periods. The patients were weighed daily before breakfast. Resting blood pressure was determined two to four times daily. In the charts of blood pressure which appear subsequently in this article the systolic and diastolic readings recorded are the lowest for each day.

Chemical and Other Methods.—Venous blood for the determination of plasma electrolytes was drawn under oil into a tube containing purified heparin. The sodium of plasma was determined by the method of Butler and Tuthill[19]; potassium by Hartzler's modification[20] of the method of Shohl and Bennett; carbon dioxide content of the plasma by the method of Van Slyke and Neill[21]; chloride by a

18. Miss Gordon Sampson, chief dietitian, Mayo Clinic metabolism unit, St. Mary's Hospital, planned and prepared the diets used in metabolism balance studies.

19. Butler, A. M., and Tuthill, E.: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material, J. Biol. Chem. **93**:171 (Sept.) 1931.

20. Hartzler, E. R.: A Note on the Determination of Potassium by the Method of Shohl and Bennett, J. Biol. Chem. **122**:19 (Dec.) 1937.

21. Van Slyke, D. D., and Neill, J. M., cited by Peters and Van Slyke,[24] p. 283.

modification of the method of Keys [22]; p_H by the glass electrode method of Dill, Daly and Forbes.[23] The blood urea was determined by the manometric method [24]; calcium by precipitation as oxalate from ashed plasma; glucose by the method of Folin and Wu [25]; inorganic phosphorus by the method of Gomori [26]; uric acid by the method of Folin [27]; alkaline phosphatase by the method of Bodansky [28]; serum albumin and globulin by an adaptation of the Kingsley biuret procedure [29]; cholesterol and cholesterol esters by the methods of Bloor [30] and Bloor and Knudson,[31] respectively; glutathione in whole blood and erythrocytes by the method of Woodward and Fry [32]; zinc sulfate turbidity of serum by the method of Kunkel.[33]

Sodium, potassium, phosphorus and calcium were determined in ashed samples of urine and homogenized suspensions of feces by the methods already mentioned. Chloride in urine and in homogenized suspension of feces was determined by a modified Volhard-Harvey titration and total nitrogen by the Kjeldahl method. Uric acid in urine was determined by the method of Brown.[34] Creatine and creatinine in urine were determined according to the method of Bonsnes and Taussky,[35] except that creatine was converted to creatinine by autoclave hydrolysis.

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22. Keys, A.: The Microdetermination of Chlorides in Biological Materials: Presentation of a Method and an Analysis of Its Use, J. Biol. Chem. **119**:389 (July) 1937.

23. Dill, D. B.; Daly, C., and Forbes, W. H.: pK′ of Serum and Red Cells, J. Biol. Chem. **117**:569 (Feb.) 1937.

24. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1932, vol. 2, p. 361.

25. Folin, O., and Wu, H.: A System of Blood Analysis: A Simplified and Improved Method for Determination of Sugar. J. Biol. Chem. **41**:367 (March) 1920.

26. Gomori, G.: A Modification of the Colorimetric Phosphorus Determination for Use with the Photoelectric Colorimeter, J. Lab. & Clin. Med. **27**:955 (April) 1942.

27. Folin, O.: A System of Blood Analysis: A Revision of the Method for Determining Uric Acid, J. Biol. Chem. **54**:153 (Oct.) 1922.

28. Bodansky, A.: Phosphatase Studies: I. Determination of Inorganic Phosphate; Beer's Law and Interfering Substances in the Kuttner-Lichtenstein Method, J. Biol. Chem. **99**:197 (Dec.) 1933.

29. Kingsley, G. R.: The Direct Biuret Method for the Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry, J. Lab. & Clin. Med. **27**:840 (March) 1942.

30. Bloor, W. R.: The Determination of Cholesterol in Blood, J. Biol. Chem. **24**:227 (March) 1916.

31. Bloor, W. R., and Knudson, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, J. Biol. Chem. **27**:107 (Oct.) 1916.

32. Woodward, G. E., and Fry, E. G.: The Determination of Blood Glutathione, J. Biol. Chem. **97**:465 (Aug.) 1932.

33. Kunkel, H. G.: Estimation of Alterations of Serum Gamma Globulin by a Turbidimetric Technique, Proc. Soc. Exper. Biol. & Med. **66**:217 (Oct.) 1947.

34. Brown, H.: Determination of Uric Acid in Human Blood, J. Biol. Chem. **158**:601 (May) 1945.

35. Bonsnes, R. W., and Taussky, H. H.: On Colorimetric Determination of Creatinine by Jaffe Reaction, J. Biol. Chem. **158**:581 (May) 1945.

Urine for determination of 17-ketosteroids and corticosteroids was collected in bottles which were kept in a refrigerator. The twenty-four hour collections were transported promptly to the laboratory. One portion was adjusted to p_H 1, allowed to stand overnight and extracted with chloroform. Corticosteroids in the extract were determined by a method which was essentially that of Corcoran and Page.[36] Another portion was acidified with one-tenth its volume of concentrated hydrochloric acid, boiled ten minutes and extracted with carbon tetrachloride. The 17-ketosteroids in the extract were determined by the method of Callow, Callow and Emmens [37] with the addition of a correction equation to compensate for overestimation due to nonketonic chromogens.[38] Urinary gonadotropins were determined by the method of Frank, Salmon and Friedman [39] and estrogens by the method of Smith and Smith.[40] Circulating eosinophils were counted according to the method originally described by Dunger [41] and modified by Forsham and co-workers.

ANTIRHEUMATIC EFFECT

It is beyond the scope of this report to describe again the dramatic alteration of the rheumatic state which rapidly follows the administration of cortisone or ACTH. This already has been done by Hench and his collaborators,[2] and a more extensive description and discussion of the phenomenon by them is soon to appear. In addition to the unpublished confirmations reported at the recent International Congress on Rheumatic Diseases by Bauer, Boland, Freyberg, Holbrook and Rosenberg,[42] confirmations by Boland and Headley,[6b] Markson,[43] Spies

36. Corcoran, A. C., and Page, I. H.: Methods for the Chemical Determination of Corticosteroids in Urine and Plasma, J. Lab. & Clin. Med. **33**:1326 (Oct.) 1948.

37. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing the Grouping—$CH_3.CO$—in Urine Extracts as Indication of Androgen Content, Biochem. J. **32**:1312 (Aug.) 1938.

38. Engstrom, W. W., and Mason, H. L.: A Study of the Colorimetric Assay of Urinary 17-Ketosteroids, Endocrinology **33**:229 (Oct.) 1943.

39. Frank, R. T.; Salmon, W. J., and Friedman, R.: Determination of Luteinizing and Follicle-Stimulating Principles in Castrate and Menopause Urine, Proc. Soc. Exper. Biol. & Med. **32**:1666 (June) 1935.

40. Smith, G. V. S., and Smith, O. W.: The Quantitative Determination of Urinary Oestrin, Am. J. Physiol. **112**:340 (June) 1935.

41. Dunger, R.: Eine einfache Methode der Zählung der eosinophilen Leukozyten und der praktische Wert dieser Untersuchung, München. med. Wchnschr. **57**:1942, 1910.

42. Bauer, W.; Boland, E. W.; Freyberg, R. H.; Holbrook, W. P., and Rosenberg, E. F.: Discussions, in the proceedings of the Seventh International Congress on Rheumatic Diseases, New York City, May 30-June 3, 1949, to be published.

43. Markson, D. E.: Prolonged Treatment of Rheumatoid Arthritis with Pituitary Adrenocorticotropic Hormone (ACTH), J. A. M. A. **141**:458 (Oct. 15) 1949.

and associates,[44] Thorn and associates[9] and Ragan, Grokoest and Boots[45] and Jonsson and co-workers[46] have been published. It is necessary only to mention the rapid regression of the articular and systemic manifestations of rheumatoid arthritis and acute rheumatic fever, loss of fever, early sense of well-being, decline of the sedimentation rate of erythrocytes and increase of appetite, weight and strength.

In the preliminary report of Hench, Kendall, Slocumb and Polley,[2a] some other effects were discussed. Others about which data were then meager were only mentioned. As a result of our subsequent observations, further effects are discussed here for the first time.

EFFECTS ON BODY WEIGHT

With few exceptions, patients who were permitted an unrestricted diet while receiving cortisone or ACTH gained moderately to markedly in weight. Usually the gain was a result of increased appetite and intake of food. In a few cases, gains and losses of weight coincided with the development and subsidence of edema. These changes in body weight will be commented on in further detail by Hench and associates.

EFFECTS ON FACIAL CONTOUR

Rounding of the facial contours was one of the earliest signs of hormonal excess.[47] As might be expected, this change came on gradually, and in any given case it was consequently difficult to date its onset. In many instances, change in facial contour was associated with considerable gain in body weight. This was not always the case, however, for definite change in facial contour was observed in 2 patients (cases 6 and 14, table 1) whose intake of food was kept at a fixed level because of metabolic studies and who consequently had gained little or no weight. Moreover, the change in facial contour could not be related to development of edema associated with retention

44. (*a*) Spies, T. D., and Stone, R. E.: Relief of the Symptoms of Acute Gout and Rheumatoid Arthritis by Means of Pituitary Adrenocorticotropic Hormone (ACTH), South. M. J. **42**:720 (Aug.) 1949. (*b*) Spies, T. D.; Stone, R. E.; Dreizen, S., and Morton, B. F.: Further Studies with Pituitary Adrenocorticotropic Hormone (ACTH), ibid. **42**:991 (Nov.) 1949.

45. Ragan, C.; Grokoest, A. W., and Boots, R. H.: Effect of Adrenocorticotropic Hormone (ACTH) on Rheumatoid Arthritis, Am. J. Med. **7**:741 (Dec.) 1949.

46. Jonsson, E.; Berglund, K.; Hävermark, N. G.; Nyström, G., and Paulsen, F.: Endocrinologic Viewpoints on Certain Rheumatic and Other Mesenchymal Diseases, Svenska läk-tidning. **37**:1921 (Sept. 16) 1949.

47. In this connection, it is perhaps significant that this is one of the earliest changes noted by many patients during the development of Cushing's syndrome due to adrenal cortical tumor or hyperplasia.

of salt and water, for the changes were sometimes observed in the absence of frank edema or persisted after edema had subsided. Nor did the faces of these patients have the appearance or feel of edema. Rather, it appeared that the altered contour probably resulted from deposition of fat in the cheeks. Changes observed in case 32 during a study of the effects of cortisone on disseminated lupus erythematosus are shown in figure 1.

EFFECTS ON THE SKIN

Acne and Hirsutism.—The possibility that cortisone might either have weak androgenic effects or might be metabolized in the body to yield androgenic compounds was suggested by the appearance of acne and hirsutism in some female patients during prolonged administration. Neither of these signs has been observed consistently or frequently, and

Fig. 1 (case 32).—(*a*) Fourteen months before administration of cortisone for disseminated lupus erythematosus was begun; (*b*) immediately before administration of cortisone was begun; (*c*) one month after having received an average of 107 mg. of cortisone daily for one hundred and sixty-six days. Between *b* and *c* there was a gain of 19.5 Kg. (43 pounds).

it would appear that they are not likely to become apparent except after prolonged administration; at least, they were observed in definite degree only in 1 woman (case 1) who received cortisone daily for a period of one hundred and eighty-seven days (fig. 2). Other evidences of androgenic activity, such as enlargement of the clitoris, masculinization of the voice and alterations in body musculature or habitus, have not been observed.

Intensification of acne which was already present occurred in a male patient, 18 years of age (case 31), who received ACTH because of acute rheumatic fever. In this case, keratosis pilaris also developed.

Keratosis Pilaris.—This relatively common cutaneous manifestation of Cushing's syndrome was observed only in case 31, just mentioned. In this case, 675 mg. of ACTH was administered in twenty-four days; subsequently, after an interval of eight days, an additional 760 mg. was administered in twenty-three days because of relapse of the acute rheumatic fever, making a total of 1,435 mg. of ACTH. Intensification of acne of the face, back, shoulders and abdomen which had been present prior to the administration of ACTH and the presence of keratosis pilaris in the same areas were first noted by the patient when he had received 225 mg. of ACTH in a period of seven days. The "sandpaper" texture of the skin gradually regressed during the twenty days that he continued under observation after administration of ACTH was

Fig. 2 (case 1).—(*a*) After the patient had received an average of 86 mg. of cortisone for eighty-three days, before obvious evidences of hormonal excess had become apparent; (*b*) after having received an average of 90 mg. of cortisone daily for one hundred and sixty-seven days; (*c*) profile view at same time as *b* showing facial hirsutism and acne.

stopped but it did not entirely disappear. While the relation of this cutaneous lesion to adrenal cortex hyperfunction is not established with certainty by the observations in this case, such a relationship seems probable.

Cutaneous Striae.—Thinning of the skin and the development of characteristic reddish or purplish striations are features of most cases of Cushing's syndrome. In the past these striae were attributed tentatively to an excessive formation of cortisone-like steroids by hyperfunctioning adrenal cortices.[14b] The proof of this was lacking until the development of distinctive cutaneous striations was observed in a

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youth 17 years old (case 25) who received 200 mg. of cortisone daily for nineteen days and 100 mg. daily for five additional days because of acute rheumatic fever. Striations were first noted over the hips and flanks at the conclusion of this period of study, that is, after he had received 4,300 mg. of cortisone in twenty-four days. During this time his weight had increased approximately 7 Kg. (3.2 pounds). The striae might have been present for some time before they were observed, as they were well developed and of a distinctive reddish purple color when first noted.

After an interval of thirteen days without treatment, administration of cortisone was resumed in this case. An additional amount of 3,100 mg. was administered in the next twenty-eight days. During this period the striae became broader and more deeply colored (fig. 3).

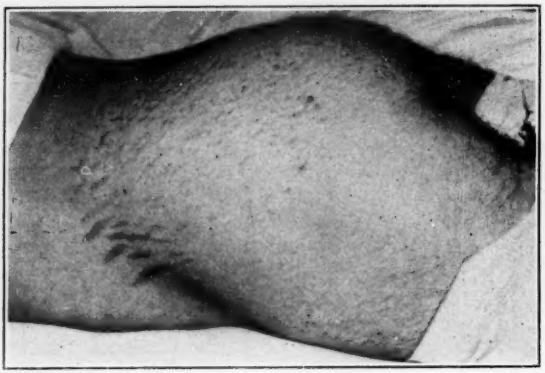

Fig. 3 (case 25).—Cutaneous striae on sixth day of the second period of use of cortisone acetate.

By the sixth day of the second period of administration of cortisone the patient's weight had increased approximately 10 Kg. since administration of cortisone was begun. He had received 800 mg. of the hormone in addition to the 4,300 mg. which he had previously received, or a total of 5,100 mg. For several weeks following the last dose of cortisone, the color of the striae faded, so that they finally resembled the frequently seen striae distensae which make their appearance in association with rapid gains of weight.

To date, the presence of striae has been observed in 5 additional patients (cases 23, 24, 26, 27 and 32), all of whom, except 1 (case 32), were young persons who received cortisone acetate. Of the 6 patients on whom striae developed, 2 were male and 4 were female. Each had gained from 3.0 to 17.7 Kg. before the first observation of the striae.

It is to be noted, however, that in each case the striae differed from ordinary striae distensae in that they were reddish or purplish.

ACTH AND MELANOPHORE HORMONE

An observation which may indicate a possible role of the pituitary gland in melanogenesis was made on 1 patient, a white man 38 years of age (case 18). During administration of ACTH to this patient because of rheumatoid arthritis and spondylitis, marked pigmentation developed in the palmar creases and the axillas, on the dorsum of the hands and feet and in two scars consequent to articular biopsies (fig. 4). There were no indications of adrenal insufficiency which might account for the development of pigmentation. The ACTH prepara-

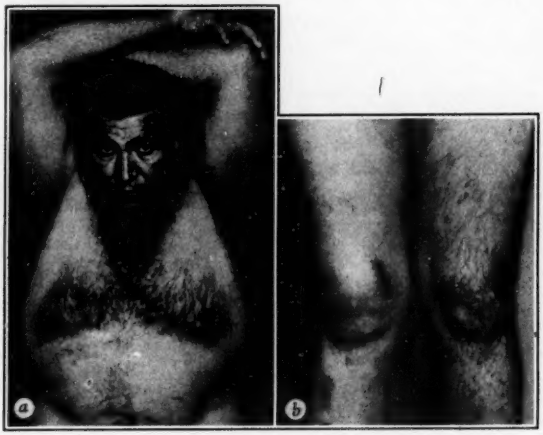

Fig. 4 (case 18).—Pigmentation (*a*) of axillas and nipples and (*b*) of scars.

tion, however, was found to contain appreciable melanophore hormone (intermedin) on bioassay in hypophysectomized frogs. This finding may be relevant to the development of pigmentation under chronic treatment with pituitary preparations.

MUSCULAR WEAKNESS

Muscular weakness has been observed in a variety of different circumstances in patients who have received cortisone or ACTH, namely, during prolonged administration of cortisone, during shorter periods of administration of ACTH and after stopping the administration of either one of these hormones. The basis for the weakness probably differs in each situation and is at present unknown.

During Administration of Cortisone.—Two patients exhibited rather marked degrees of weakness during administration of cortisone after they had received the hormone for prolonged periods. One of these, a man, 49 years of age (case 16), who had reecived cortisone daily for several weeks because of severe rheumatoid arthritis, became so weak that he was unable to step up on a low bedside stool unaided. With prolonged administration of the hormone, partial relapse of the rheumatoid arthritis, associated with marked elevation of the sedimentation rate of erythrocytes, developed. Some of his weakness may have been related to the arthritis and to enforced inactivity, rather than to the effects of the hormone itself. The level of the serum potassium remained normal in this case, and the weakness was not significantly modified by the administration of potassium chloride or testosterone propionate.

Another patient, a woman 29 years of age (case 1), with rheumatoid arthritis, received cortisone daily for one hundred and eighty-seven days. During most of this period, the daily dose was 100 mg. Weakness, principally involving the legs, was complained of and became objectively apparent approximately three and a half months after the administration of cortisone was begun. Subsequently the weakness became more marked, its progression coinciding with the development of other manifestations of Cushing's syndrome, including amenorrhea, facial acne, facial hirsutism, mental depression and impairment of glucose tolerance. There was no correlation between the development of weakness and the level of the serum potassium, the lowest value observed being 3.4 milliequivalents per liter. Metabolic balance studies were carried out during the last six days of administration of cortisone and for twelve days following its cessation. The data obtained during this period of observation indicated that the patient was not in negative nitrogen or potassium balance. Moreover, she did not retain significant amounts of nitrogen or potassium after withdrawal of cortisone. The latter observation suggests that there had not been a serious depletion of body protein or potassium during the long period of cortisone administration. The physiologic basis for the weakness in this case, therefore, was not readily apparent. Its onset before the patient's physical activity was seriously limited by mental depression would seem to indicate that it was not explainable solely by disuse of muscles.

During and After Administration of Cortisone and ACTH.—Another patient, a woman aged 45 years (case 14), with rheumatoid arthritis, exhibited moderate muscular weakness during and following administration of both cortisone and ACTH. The occurrence of weakness coincided with depression of the level of serum potassium to as low as 2.54 milliequivalents per liter during administration of 100 mg. of ACTH daily and to as low as 2.0 milliequivalents per liter during

administration of 200 mg. of cortisone daily. The results of metabolic studies in this case are given subsequently. During the period of hypopotassemia, characteristic alterations of the electrocardiogram were observed. In this case it seems likely that weakness was related, at least in part, to hypopotassemia. This possibility could not be tested by administration of potassium salts because of metabolic studies which were in progress.

Varying degrees of asthenia were observed in several patients within a few days after the administration of cortisone was stopped. This was most pronounced in cases 3, 5 and 14. The duration of asthenia varied from a few days to several weeks. It was never totally incapacitating. Its severity and duration tended to be greatest after prolonged periods of administration of cortisone. It was not associated with significant abnormalities of the blood electrolytes or with hypoglycemia. Unequivocal evidence of severe adrenal cortex insufficiency was not present. There was, however, some evidence of depression of adrenal cortex function and, as will be discussed later, this may have contributed to the asthenia.

EFFECT ON MENSTRUAL FUNCTION

In the group of patients studied were 10 women who had normal or almost normal menstrual function prior to the administration of cortisone or ACTH. Of the 10 women, 6 received cortisone only, 1 received ACTH only, 2 received both cortisone and ACTH and 1 received cortisone, compound F and ACTH. In no case in which more than one hormone was employed were they administered concurrently.

Of the 10 women, 3 (cases 1, 24 and 27) became amenorrheic during the period of administration of cortisone. A fourth (case 29) missed a period which was due a few days before the administration of ACTH was started. She did not menstruate either during administration of ACTH or for a prolonged interval thereafter, although recovery from acute rheumatic fever seemed complete. Three of the 4 who became amenorrheic were young girls who had been menstruating three years or less (cases 24, 27 and 29). The data relative to menstrual function in this group of patients, including those who did and did not become amenorrheic, are summarized in table 2. The data given in this table can be supplemented with the following details concerning the 4 cases in which amenorrhea developed.

CASE 1.—This patient had a past history of menstrual irregularity, the interval between periods having been occasionally as short as two weeks and as long as six weeks. Duration of flow was usually three to five days. The two periods prior to the onset of amenorrhea had been normal. The last period began on the ninety-third day of administration of cortisone, after the patient had received

a total of 7.25 Gm. of the hormone, or an average daily dose of approximately 79 mg. After this, menstruation did not occur during an additional ninety-five days of administration of cortisone, during which time she received 9.825 Gm. of the hormone, or for approximately six months after administration of cortisone was stopped. Menses were then resumed at irregular intervals. Examination during the period of administration of the hormone disclosed no signs of pregnancy or pelvic abnormality.

CASE 24.—This patient, a girl 15 years old, had no antecedent history of menstrual abnormality. The onset of acute rheumatic fever occurred on March 24, 1949. Administration of cortisone was started on March 28. She received, in all, 4.65 Gm. in thirty-eight days, or an average daily dose of approximately 122 mg. Bleeding began on April 18, while she was receiving cortisone. Administration of the hormone was concluded on May 4. There was no menstruation during the

TABLE 2.—*Menstrual Function in a Group of Ten Women With Rheumatoid Arthritis or Acute Rheumatic Fever Who Received Cortisone, ACTH or Compound F*

					Hormones Administered			
Case	Age, Yr.	Marital State	Previous Menstrual History	Previous Pregnancies, Number	Name	Days	Total Amount, Gm.	Alteration in Menstrual Function
1	29	M	Abnormal*	2	Cortisone	187	17.075	Amenorrhea†
6	49	M	Abnormal‡	6	Cortisone	10	1.0	None
					ACTH	12	1.2	None
					Compound F	12	0.9	None
					Cortisone	30	4.2	None
9	44	M	Normal	2	Cortisone	25	2.15	None
11	40	S	Normal	0	Cortisone	8	1.0	None
					ACTH	12	1.26	None
					Cortisone	18	3.6	None
13	32	M	Normal	1	Cortisone	29	3.7	None
21	30	M	Normal	1	Cortisone	56	5.6	None
24	15	S	Normal	0	Cortisone	38	4.65	Amenorrhea†
27	14	S	Normal	0	Cortisone	40	5.2	Amenorrhea†
29	13	S	Abnormal§	0	ACTH	38	0.825	Amenorrhea†
33	25	S	Normal	0	Cortisone	43	4.5	None
					ACTH	28	3.6	None

* The interval varied from two to six weeks.
† Details of development of amenorrhea in relation to administration of hormones are given in the text.
‡ Menses were irregular during the preceding year.
§ The period which was due two days before administration of ACTH was begun did not occur.

month of May, but a scanty period began on June 17. Two other periods, not normal in amount, but each less scanty than the preceding one, occurred in the middle of July and the middle of August. In September the period was normal.

CASE 27.—The patient, a girl 14 years old, had been menstruating normally for approximately two years. She had experienced attacks of acute rheumatic fever in 1938, 1942 and 1945. The most recent attack began on April 26, 1949. A normal menstrual period had begun on April 23. Administration of cortisone was started on May 12. In forty days the patient received a total of 5.2 Gm. of the hormone, or an average daily dose of 130 mg. Menses did not occur during the period of administration of cortisone or for one hundred and ten days after administration of the hormone was stopped. Normal menses were then resumed.

CASE 29.—The patient, a girl 13 years old, had her first menses five months before acute rheumatic fever developed on May 31, 1949. Administration of ACTH was begun on June 10 and discontinued on July 17. A total of 0.825 Gm.

was administered in thirty-eight days or an average daily dose of approximately 22 mg. Symptoms and signs of acute rheumatic fever subsided. A menstrual period began on May 8, 1949. A period which was due on or about June 8, just before the administration of ACTH was begun, did not occur. Menstruation did not occur during the period of administration of ACTH, nor had it been resumed on December 1, when the patient last reported.

Factors Responsible for Amenorrhea.—Consideration must be given to the possibility that the amenorrhea in these 4 cases was an effect of a severe disease rather than some alteration produced by the hormones administered. Although the former possibility cannot be excluded with certainty in any of the cases, and particularly in case 29, it seems unlikely because 3 patients (cases 1, 24 and 27) were improving or apparently cured when amenorrhea developed. In case 29 amenorrhea persisted long after recovery from acute rheumatic fever. Furthermore, the common occurrence of amenorrhea in states of chronic excess of adrenal cortex hormones, such as Cushing's syndrome, creates a presumption that the amenorrhea in these 4 cases was hormonally induced or maintained.

Assuming that the amenorrhea in these cases actually was hormonally induced, we should consider briefly the physiologic mechanisms involved. In no case was cessation of menstruation accompanied with vasomotor symptoms. The occurrence of amenorrhea in only 1 woman, and in her case only after the daily administration of cortisone for more than three months, suggests that menstrual function in the mature woman is not easily disturbed by ACTH and cortisone. On the other hand, the occurrence of amenorrhea in 3 adolescent girls after relatively short periods of administration of cortisone or ACTH suggest that menstrual function in young persons is more easily disturbed by these hormones.

To date, our limited efforts to study the effects of cortisone and ACTH on menstrual function by means of bioassay of the urine for estrogens and gonadotropins and by endometrial biopsy and vaginal smears have not been fruitful. Such studies were not carried out on the 4 subjects who had amenorrhea. The following observations on other patients are recorded, although by themselves they contribute little to an understanding of the problem: 1. A menopausal woman, 43 years of age (case 12), continued to experience severe hot flashes during administration of 5.9 Gm. of cortisone in fifty-seven days. This suggests that cortisone did not seriously impair the gonadotropic function of the anterior lobe of the pituitary gland during this time, inasmuch as hot flashes are usually associated with elevated urinary excretion of pituitary gonadotropins. Subsequently, hot flashes did not occur during simultaneous administration of 100 mg. of cortisone and 5 mg. of estrone twice weekly. 2. In a woman, aged 49 years (case 6), whose

menstrual periods had been irregular for one year, increasing estrogenic activity was demonstrated in the urine, from 0 up to 230 rat units per twenty-four hours during the daily administration of 100 mg. of ACTH for twelve days. In another woman, 45 years of age (case 14), in whom menopause had been induced four years previously by roentgen therapy of the ovaries, estrogenic activity of the urine increased from 0 to 10 rat units at the conclusion of administration of 100 mg. of ACTH daily for twelve days. The origin of the increased urinary estrogens in these 2 cases could be either the adrenal cortices, owing to stimulation by ACTH, or the ovaries, owing to stimulation by gonadotropin which might have been present in the preparation of ACTH. However, the latter possibility is less likely, inasmuch as the preparation of ACTH in question was tested for gonadotropic activity in both normal and hypophysectomized female rats. After a dose of 8 mg. no gonadotropic activity was demonstrated. 3. In the case of a woman 30 years of age (case 21), the excretions of estrogens and gonadotropins and the menstrual periods were entirely normal for two menstrual cycles during which time she received 100 mg. of cortisone daily. 4. A woman, 32 years of age (case 13), became pregnant at approximately seven weeks after she had received 3.7 Gm. of cortisone in twenty-nine days. There had been one other pregnancy, seven years previously. The patient stated that she had been trying for three years to become pregnant again.

EFFECTS ON LIBIDO IN THE MALE

The evidence relative to the effect of cortisone and ACTH on sexual function in the male is inconclusive but suggests that cortisone, at least, may cause a diminution of libido in some cases. The common occurrence of impotence, presumably of hormonal origin, in men with Cushing's syndrome suggests that this symptom might occur frequently among men who received cortisone for prolonged periods.

Systematic inquiry about libido in 5 cases (cases 2, 3, 4, 10 and 15) revealed that in 3 (cases 4, 10 and 15) there was apparently a significant decrease in sexual drive and potency during prolonged periods of administration of cortisone. One of the 3 patients (case 15) also noted a similar change while receiving ACTH. A fourth patient, 64 years of age (case 3), felt that libido had not been depressed but, indeed, might have improved slightly during administration of cortisone. The fifth, 41 years of age (case 2), had noted a decline in libido with progression of the symptoms of rheumatoid arthritis and thought that there was a further decline while he was in the hospital. Systematic inquiry was not made in other cases in which the complaint was not offered voluntarily. It is apparent that additional data are needed to permit evaluation of hormonal effects on this complex function.

HORMONALLY INDUCED "EUPHORIA" AND DEPRESSION

Observations on the effects of cortisone and ACTH on the psychic state will be presented only briefly. They are to be reported in detail by our colleagues in the section on psychiatry, Drs. F. J. Braceland and H. P. Rome.

The striking sense of well-being exhibited by many patients early in the period of administration of cortisone or ACTH provoked questions by Hench and co-workers [28] and others concerning the capacity of these hormones to induce a state of euphoria beyond what might reasonably be expected consequent to abrupt improvement in their physical condition. The problem is a complex one. Obviously, the answer to these questions depends in part on a definition of the term "euphoria." A sense of well-being and apparent acceleration of mental activity might be anticipated during a rapid regression of a chronic, painful, debilitating, disabling malady, such as rheumatoid arthritis, even if the agent which was responsible for the regression of the disease was totally lacking in direct effects on the central nervous system. We will assume for the moment that a distinction can and should be made between altered emotional reactions related to improved physical health and those which are not so related and will apply the term "euphoria" here only to the latter.

Preliminary observations by Braceland and Rome [48] have suggested that emotional alteration is most strikingly observed in those patients who experience the most abrupt and dramatic improvement in their physical condition. Contrariwise, "euphoria" tends to be less evident when physical improvement is less dramatic. On the basis of this gross evidence alone, it therefore, would appear that the degree of euphoria bears a direct relationship to the physical state of the patient prior to administration of the hormone and the rapidity with which it is changed for the better.

Nevertheless, a clinical impression, ours and that of others, remains to support the suggestion that cortisone and ACTH actually exert some euphorogenic effect as previously defined.[49] In addition to our own cases, there is the case of Rawson,[50] for example, in which a man, severely ill with advanced and terminal myelogenous leukemia, experienced such a pronounced acceleration of mental processes during administration of ACTH that for the first time in months he began making constructive plans for the pursuit of his scientific work. There was no accompanying

48. Braceland, F. J., and Rome, H. P.: Unpublished observations.

49. It is perhaps relevant here that Kepler (personal communication), while recognizing the common occurrence of mental depression in patients with Cushing's syndrome, commented that occasionally garrulousness, hyperactivity and euphoria are encountered..

50. Rawson, R. W.: Personal communication to the authors.

improvement in the leukemia, which, indeed, terminated fatally within a week.

There is as yet, however, no good evidence that these hormones are euphorogenic agents which might be useful in the treatment of mental disease characterized by depression. This tentative conclusion is further supported by the studies of Braceland and Rome [48] on a small group of patients suffering from depression, paranoid schizophrenia and catatonic schizophrenia.

Mental depression of significant degree, similar to that observed in some cases of Cushing's syndrome, developed in 1 case (case 1) in which cortisone was given daily for one hundred and eighty-seven days. The patient was a woman, 29 years of age, with severe rheumatoid arthritis. She had not previously been nervously depressed more than might have been expected as a reaction to prolonged illness. During the early weeks of administration of the hormone, she had maintained an exceptionally cooperative and contented attitude suggestive of mild "euphoria." A change in mood first became clearly apparent after she had received injections of the hormone for approximately nineteen weeks. During this period she received a total of 11.475 Gm., or an average daily dose of approximately 86 mg. With continued administration of the hormone, the depression, together with other evidence of Cushing's syndrome, became progressively more marked. When administration of the hormone was stopped, the features of Cushing's syndrome, including the depression, regressed rather rapidly, although severe manifestations of rheumatoid arthritis quickly recurred.

The characteristics of the depression in this case will be described in further psychiatric detail by Braceland and Rome.[51] Suffice it here to say that from a psychiatric standpoint it did not have the characteristics of a reactive depression consequent to prolonged illness and hospitalization. That it probably represented a hormonal effect is suggested by the following facts: 1. The patient was a reasonably stable person. 2. Depression developed in spite of improvement in her articular disease. 3. It regressed promptly when use of cortisone was discontinued, in spite of a rapid increase in the severity of the arthritis.

EFFECTS ON BLOOD PRESSURE

With one exception, changes in blood pressure during administration of cortisone or ACTH were slight or absent in our 33 cases. As examples are the observations on blood pressure in cases 5, 14, 11 and 6 included in figures 9, 12, 15 and 18, respectively, in the section dealing with metabolic and blood chemical studies. In each of these the blood pressure was normal before administration of the hormones and remained so.

51. Footnote deleted.

CASE 32.—The only patient who exhibited a significant rise in blood pressure while receiving cortisone was a woman, 32 years of age, who was severely ill with disseminated lupus erythematosus. At the time of admission to the hospital the blood pressure was 124 mm. of mercury systolic and 80 diastolic on one occasion and 132 systolic and 80 diastolic on another. The urine contained erythrocytes, casts and albumin, but the concentration of blood urea was normal at the time and on several subsequent occasions. However, five months prior to admission, she had experienced during pregnancy an acute episode which in retrospect may have represented disseminated lupus erythematosus with renal involvement.

This patient was given a total of 6.475 Gm. of cortisone acetate in fifty-eight days. Her general condition improved greatly, but cutaneous striae and rounding of facial contours developed (fig. 1). The blood pressure then was found to be 188 mm. of mercury systolic and 116 diastolic. The ocular fundi, which had not been examined previously, showed slight narrowing of the retinal arterioles. Cortisone was administered in a dose of 100 mg. daily for another one hundred and thirteen days. During this time, the blood pressure varied from 146 mm. of mercury systolic and 94 diastolic to 194 systolic and 122 diastolic. Headaches at the vertex suggestive of those of hypertensive origin occurred at intervals. Twelve days after administration of cortisone was stopped, the blood pressure varied from 104 to 132 mm. of mercury systolic and 74 to 100 diastolic.

It is apparent from the foregoing brief summary of the data in this series that, with the exception of the case just cited, in which the patient probably had parenchymatous renal disease, cortisone and ACTH had little effect on blood pressure. This does not rule out the possibility (and, indeed, suggests it) that these hormones may induce significant hypertension in certain circumstances, for example, in the presence of renal disease.

EFFECTS ON URINARY STEROIDS

Determinations of urinary corticosteroids and 17-ketosteroids [52] were made before, during and after administration of hormones in the majority of our 33 cases.[53]

Corticosteroids.—In figure 5 are shown values for the urinary corticosteroids during a protracted period of administration of cortisone to a woman (case 1) with rheumatoid arthritis. During administration of 100 mg. of cortisone daily the values, with few exceptions, were maintained at levels distinctly above the one control value (1.2 mg. per twenty-four hours). Toward the end of the period of administration of 100 mg. of cortisone daily elevated values also were obtained. After adminstration of the hormone was stopped, the values diminished

52. The normal values for 17-ketosteroids in our laboratory are as follows: for men 6 to 20 mg. per 24 hours, average 14; for women, 4 to 17 mg. per 24 hours, average 10; for corticosteroid in both men and women 0.3 to 1.2 mg. per 24 hours, average 0.56.

53. Details of these and other relevant studies are to be reported by Mason, H. L.: Isolation of Adrenal Cortical Hormones from Urine: 17-Hydroxycorticosterone and 17-Hydroxy-11-Dehydrocorticosterone, J. Biol. Chem.

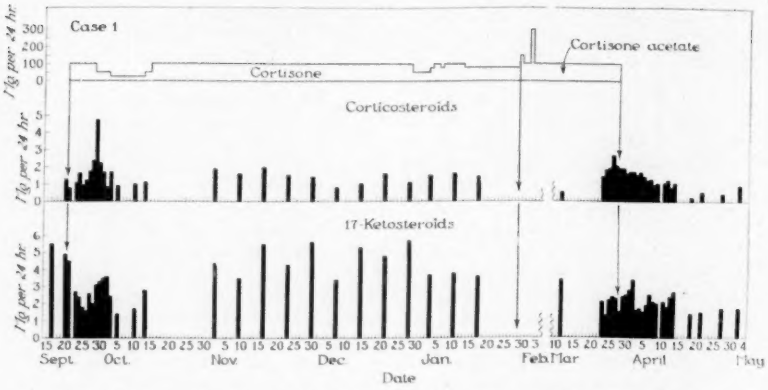

Fig. 5 (case 1).—Urinary corticosteroids and 17-ketosteroids before, during and after prolonged administration of cortisone. With prolonged administration of the hormone the excretion of 17-ketosteroids dropped to low levels, which persisted after administration was stopped.

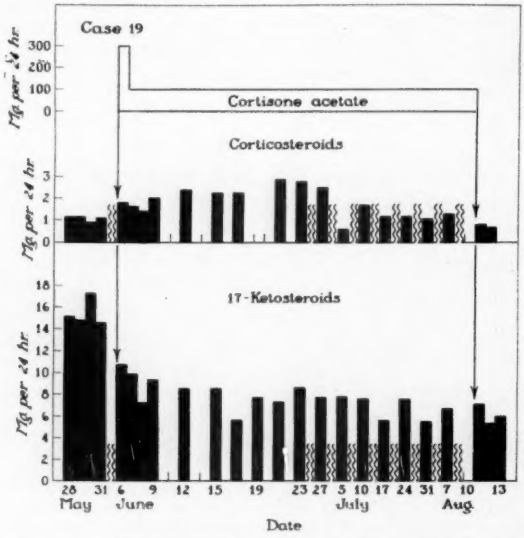

Fig. 6 (case 19).—Urinary corticosteroids and 17-ketosteroids before, during and after administration of cortisone for sixty-six days. Note the prompt decrease in 17-ketosteroids when administration of the hormone was begun.

slowly; this suggests that cortisone was still present at the sites of injection and was being slowly absorbed.

The values for corticosteroids shown in figure 6 (case 19) increased to almost 3 mg. per twenty-four hours after 100 mg. of cortisone had been administered daily for approximately two weeks. Thereafter they decreased to between 1.0 and 1.5 mg. per twenty-four hours. Immediately after administration of the hormone was stopped they were within the normal range of values.

Prolonged elevation of the values of urinary corticosteroids after administration of cortisone was stopped in case 14 is seen in figure 7.

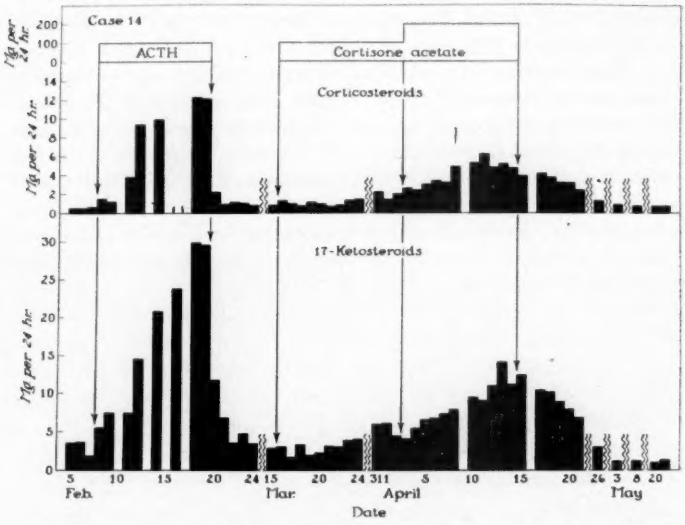

Fig. 7 (case 14).—Influence of ACTH and cortisone on urinary excretion of corticosteroids and 17-ketosteroids. The high levels attained during administration of ACTH and the relatively high levels during and for at least a week following administration of 200 mg. of cortisone daily should be noted. Subsequently the 17-ketosteroids decreased to low levels.

In this case the patient received 100 and then 200 mg. of cortisone daily. During the period of administration of 100 mg. daily, the corticosteroid values did not differ significantly from the control level until toward the end of the period. However, when the dose was increased to 200 mg. daily the values increased to more than 5 mg. per twenty-four hours.

The pronounced effect of ACTH on the excretion of corticosteroids in a case of rheumatoid arthritis (case 14) is also illustrated in

figure 7. A similar prompt increase with attainment of maximal values in nine to eleven days was observed in 3 other cases. In 2, in which the patients were women (cases 6 and 11) and in which 100 to 105 mg. of ACTH was given daily, the maximal values were 17.5 and 7.0 mg. per twenty-four hours, respectively. In the third case, that of a man (case 17), the excretion reached a maximum of 5.7 mg. in twenty-four hours after 70 mg. of ACTH was given daily. In all 4 of these cases 17-hydroxycorticosterone (compound F) was isolated in amounts that were 30 to 50 per cent of the total corticosteroids. Thus, the foregoing observations suggest that stimulation of the adrenal cortex with ACTH promotes secretion of 17-hydroxycorticosterone (compound F) rather than cortisone. Small amounts of cortisone were isolated also from the urine of patients who were given cortisone.

17-Ketosteroids.—It was observed repeatedly that when the values for urinary 17-ketosteroids were originally at a normal level, administration of cortisone or its acetate was followed by a prompt and striking fall in the 17-ketosteroid values (figs. 5 and 6). After the initial fall there was an increase (fig. 5) or a leveling off of the 17-ketosteroid values (fig. 6). During prolonged treatment the values gradually diminished to low levels (fig. 5) which persisted for several weeks at least after administration of cortisone was stopped. This latter effect also is seen in figure 7.

When the initial level of 17-ketosteroids was very low, the level of 17-ketosteroid sometimes gradually increased during administration of cortisone (fig. 7). Noteworthy is the increase of the 17-ketosteroid values to more than 14 mg. per twenty-four hours when 200 mg. of cortisone was given daily.

The persistently low levels which followed prolonged administration of cortisone are suggestive of depression of some functions of the adrenal cortex at least. It is believed that the initial fall from normal levels indicates a suppression of cortical function and that the 17-ketosteroids excreted during administration of cortisone are derived largely, if not entirely, from the cortisone administered. This belief is supported by unpublished data on isolation of steroids from the urine. The 17-ketosteroids, androsterone and etiocholanolone which are ordinarily found in urine and which in women are products of adrenal cortex function, could not be found. Other ketosteroids have been isolated but not identified as yet.

The maximal excretion of 17-ketosteroids during administration of ACTH varied from 21 to 45 mg. per twenty-four hours in the 4 cases mentioned previously. The prompt and very pronounced increase in the excretion of 17-ketosteroids in case 14 is illustrated in figure 7.

METABOLIC AND BLOOD CHEMICAL STUDIES

The metabolic studies had three principal objectives: (1) to elucidate the mechanism of action of cortisone and ACTH in rheumatoid arthritis; (2) to secure basic data on the metabolic effects of these substances in man, and (3) to learn something of the possible hazards involved in the administration of these hormones. The first objective has not been attained; the second and third have been attained at least in part.

Balance studies for nitrogen, calcium, phosphorus, potassium, sodium and chloride were carried out. The changes in blood electrolytes were followed. The urinary excretions of uric acid, creatine and creatinine were determined. Blood chemical determinations which were performed on many of the patients included those for albumin, globulin, cholesterol, cholesterol esters, alkaline phosphatase, calcium, phosphorus and uric acid. A series of determinations of the basal metabolic rate was made in 5 cases. Periodic estimations of the blood sugar were made in most cases. Glutathione in erythrocytes and whole blood was determined at intervals in 3 cases. Estimations of zinc sulfate turbidity in serum were carried out in several cases.[54]

Balance Studies.—For purposes of metabolic study, cortisone was administered in doses of 100 and 200 mg. daily in two doses and ACTH in doses of 100 and 105 mg. daily in three or four doses. The influence of simultaneous administration of estrone and of testosterone propionate on the metabolic effects of cortisone was studied in 1 patient. The effects of 900 mg. of 17-hydroxycorticosterone (compound F) administered in a period of twelve days were studied in 1 patient. There was no apparent correlation between the metabolic effects produced by the hormones and the clinical response of the arthritis.

CASE 5.—Alterations in the concentrations of the plasma electrolytes in the case of a man, 34 years of age, with rheumatoid arthritis of four and a half months' duration, who received 100 mg. of cortisone daily for five metabolic periods of six days each are shown in figure 8. Corresponding balance data are shown in figure 9. Only slight changes in the plasma electrolytes were observed; a mild alkalosis developed during administration of cortisone. The level of the plasma potassium did not decrease significantly. (In several other cases in which 100 mg. of cortisone was administered daily for varying periods, there were, as a rule, minimal or no changes in the plasma electrolytes. Mild lowering of the plasma potassium was occasionally encountered.)

During the first three periods of administration of the hormone (periods 4, 5 and 6, fig. 9), there was a gradual increase in the excretion of sodium and chloride, so that the balances became negative in the third period (that is, period

54. It will be apparent that some of the determinations were performed for the purpose of finding suggestive leads for more intensive study in the future. Also, it will be apparent that in the present brief presentation of data on metabolic balances only the more conspicuous or clinically significant aspects can be emphasized

6). In the fourth and fifth periods of administration of cortisone (that is, periods 7 and 8), the balances for sodium and chloride again became positive, as they had been in the three control periods prior to administration of the hormone. There was a slight increase in nitrogen excretion (fig. 10), the balance becoming negative in the fourth and fifth periods of administration of the hormone (that is in periods 7 and 8). Changes in the excretion of calcium and phosphorus were probably not significant, although it is suggested that cortisone produced an increase in fecal calcium and phosphorus. This suggestion is supported by similar observations of

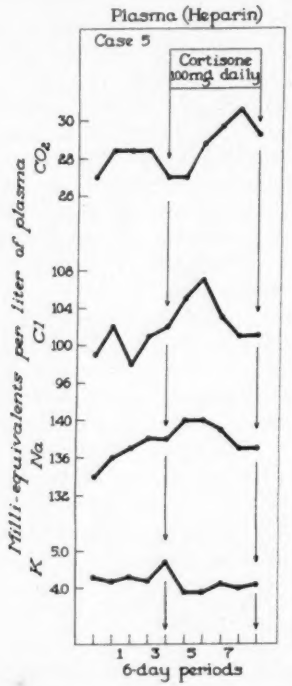

Fig. 8 (case 5).—Minimal alteration of plasma electrolyte pattern during administration of 100 mg. of cortisone daily. A mild alkalosis developed.

more definite degree in later studies on other patients (balance data in cases 6 and 14).

In general, it can be said that in this patient the observed metabolic effects of cortisone in a dose of 100 mg. daily for the thirty days which were studied were not pronounced.

CASE 4.—An additional metabolic study of the effects of 100 mg. of cortisone daily for twelve days was carried out on a man, 41 years of age, with chronic

rheumatoid arthritis of two years' duration. In view of the essentially negative character of the data in this case and in order to conserve space, figures and charts will not be presented. No significant changes in the concentrations of the plasma electrolytes occurred in this case. There was a slight increase in the retention of

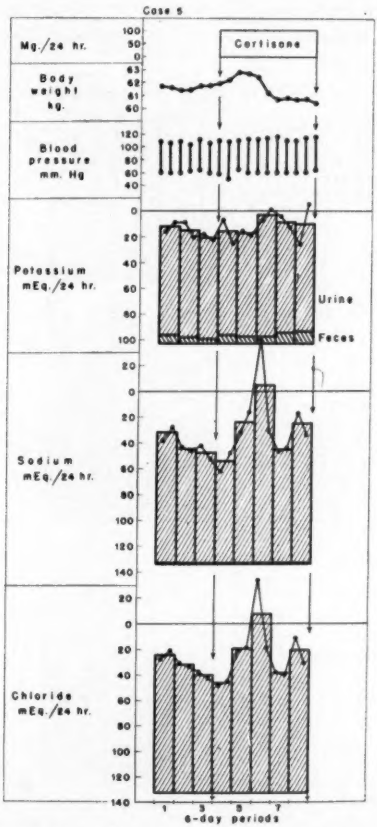

Fig. 9 (case 5).—Body weight, blood pressure and balance data for potassium, sodium and chloride. In this and subsequent illustrations showing balance data, the daily intake is charted from the 0 line downward and the average daily excretion (feces below, urine above) from the bottom line upward. A negative balance is therefore indicated by extension of the column above the 0 line, and a positive balance, by a clear area below the 0 line. Because the urinary excretion of electrolytes in some instances varied so widely from day to day within a single six-day period, the figures for daily urinary excretion of potassium, sodium and chloride are shown in some instances and in others the mean values for three days.

sodium and chloride in the second six days of administration of the hormone. No significant changes in the balances of nitrogen, potassium, calcium and phosphorus occurred.

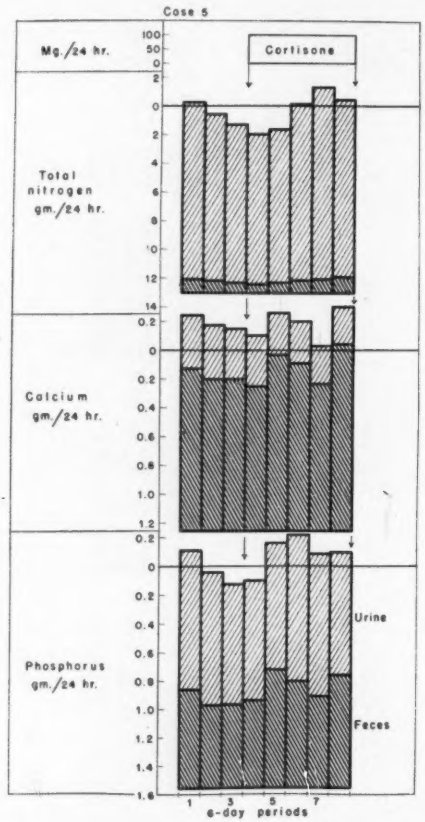

Fig. 10 (case 5).—Balance data for nitrogen calcium and phosphorus.

It can be concluded that in this case, as in case 5, cortisone in a dose of 100 mg. daily did not have pronounced metabolic effects, so far as they were measured. The clinical effects of cortisone on the rheumatoid arthritis in the 2 cases, on the other hand, were dramatic, as described by Hench, Kendall, Slocumb and Polley [2a]

CASE 14.—The patient was a woman, 45 years of age, who had moderately severe chronic rheumatoid arthritis for five years. Artificial menopause had been induced four years previously by roentgen therapy of the ovaries.

Changes in the plasma electrolytes and p_H of the blood during and after administration of several hormones in this case were more pronounced than in any of the other cases studied. The alterations which occurred during and after administration of ACTH, cortisone, cortisone plus estrone and cortisone plus testosterone propionate are shown in figure 11. Corresponding balance data for electrolytes are shown in figure 12.

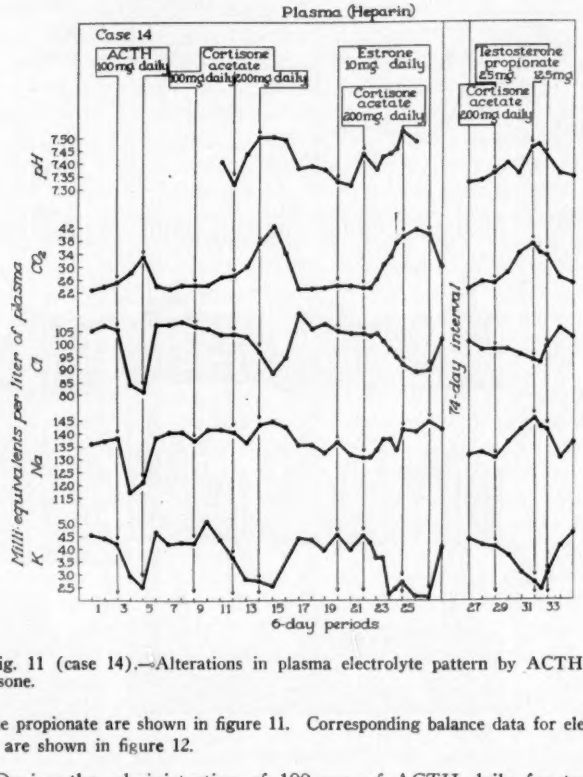

Fig. 11 (case 14).—Alterations in plasma electrolyte pattern by ACTH and cortisone.

During the administration of 100 mg. of ACTH daily for twelve days, the values for plasma bicarbonate increased, those for potassium, and sodium decreased and those for chlorides decreased markedly. Plasma electrolytes were determined at intervals of six days, and the most marked changes were observed at the end of the period of administration of ACTH. Six days after doses of ACTH were stopped, the concentrations of plasma electrolytes had returned to normal. Changes

in balances of potassium, sodium and chloride, together with changes in blood pressure and body weight during the development of the foregoing changes in the plasma electrolytes, are shown in figure 12. On the first two days of administration of ACTH, marked retention of sodium and

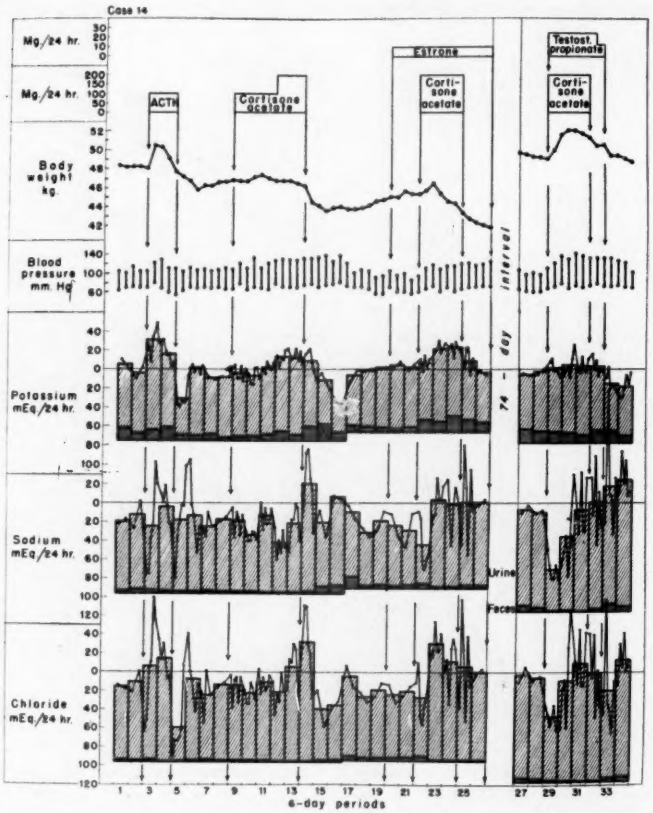

Fig. 12 (case 14).—Body weight, blood pressure and balance data for potassium, sodium and chloride.

chloride and slight loss of potassium were noted. Subsequently, the excretion of sodium, potassium and chloride increased above the control levels so that the net balances of the latter two ions were markedly negative during the twelve days of administration of ACTH. With cessation

of administration of ACTH, there was marked retention of chloride and potassium and slight retention of sodium which compensated for the previous loss of these electrolytes.

In a subsequent study, cortisone was administered in a dose of 100 mg. daily for eighteen days, followed immediately by 200 mg. daily for twelve days. Changes in the plasma electrolytes during eighteen days when the dose was 100 mg. daily were slight (fig. 11). However, during and after the administration of the hormone in a dose of 200 mg. daily, a rather severe hypochloremic, hypopotassemic alkalosis developed, similar in all respects to the alkalosis which is observed in some cases of Cushing's syndrome. The most marked changes were observed twelve days after administration of the hormone was stopped. Subsequently, the values gradually reverted to normal. The highest p_H of blood observed was 7.50. In contrast to the decrease in plasma sodium which occurred during administration ACTH, there was a slight increase in plasma sodium following administration of cortisone. As a consequence of this, there was a more marked rise in plasma bicarbonate under the influence of cortisone than had previously been observed under the influence of ACTH. During or at the end of the thirty days of administration of cortisone, negative balances for potassium, chloride and sodium, in that order of magnitude, eventually developed (fig. 12). The loss of sodium did not become apparent until the first six day period after administration of the hormone was stopped. The excretion of chloride, on the other hand, increased slightly beyond the intake in the last period of administration of cortisone (that is, period 13), and the chloride balance became more markedly negative in the first period after cessation of the administration of the hormone (that is, period 14). On the basis of other observations concerning the duration of effect of cortisone after cessation of administration, it seems likely that the loss of sodium and chloride in period 14 was probably due to a continuing hormonal effect rather than to withdrawal of the hormone. This view is supported by the retention of sodium and chloride which occurred in period 15, when the physiologic effect of the hormone was presumably waning.

The effects of 200 mg. daily of cortisone on the concentrations of the plasma electrolytes and on electrolyte balance were not modified by the prior and simultaneous administration of estrone in a dose of 10 mg. daily (figs. 11 and 12).

When cortisone in a dose of 200 mg. daily and testosterone propionate in a dose of 25 mg. daily were administered simultaneously to the patient, the loss of nitrogen which was previously observed during administration of cortisone alone (discussed in the next paragraph) did not occur and the loss of potassium was less than had previously been observed with cortisone alone. Nevertheless, the devel-

opment of a hypochloremic, hypopotassemic alkalosis under the influence of cortisone was not prevented by testosterone in this dosage (fig. 11). Subsequent studies are planned in an effort to determine whether or not maintenance of potassium balance by the administration of larger doses of testosterone propionate concurrently with cortisone would prevent the development of this type of alkalosis.

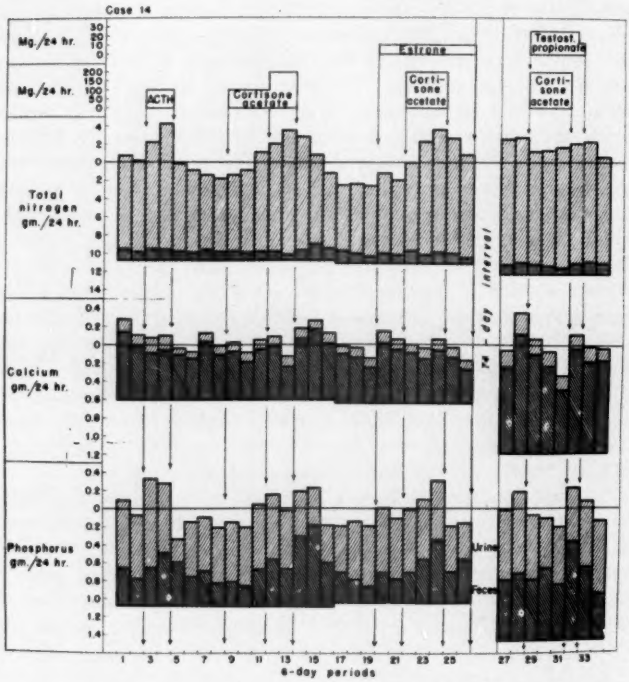

Fig. 13 (case 14).—Balance data for nitrogen, calcium and phosphorus.

Balance data for nitrogen, calcium and phosphorus are shown in figure 13. A negative nitrogen balance of considerable magnitude was observed during administration of ACTH, cortisone and cortisone plus estrone. As mentioned previously, an increase in excretion of nitrogen was prevented by the simultaneous administration of testosterone propionate. Changes in urinary calcium and phosphorus were generally slight or absent during administration of cortisone. Significant increases in urinary calcium and phosphorus occurred during

administration of ACTH. The data indicate that there was a significant increase in fecal phosphorus during and after all four programs of treatment.

CASE 11.—The patient, a woman 40 years of age, had moderately severe chronic rheumatoid arthritis of five years' duration. Observations on the behavior of the plasma electrolytes and balance data for electrolytes in this case during administra-

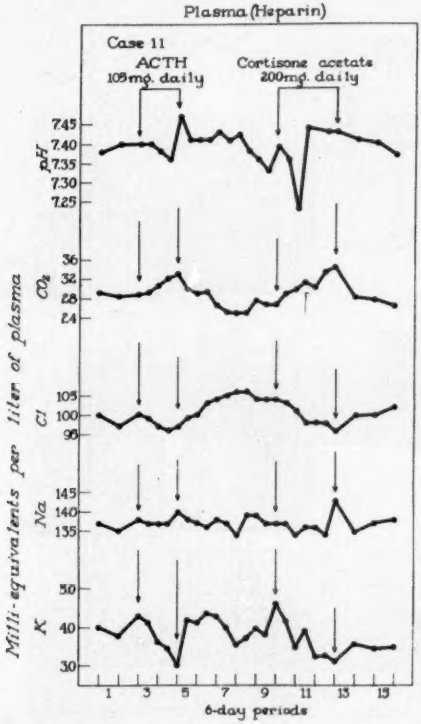

Fig. 14 (case 11).—Alterations in plasma electrolyte pattern by ACTH and cortisone.

tion of ACTH in a dose of 105 mg. daily for twelve days and cortisone in a dose of 200 mg. daily for eighteen days are shown in figures 14 and 15. Changes observed were, in general, similar to those observed in case 14, but of somewhat smaller magnitude. During and after administration of ACTH and subsequently during and after administration of cortisone, hypochloremic, hypopotassemic alkalosis developed. During the first two periods of administration of cortisone, there was retention of sodium and chloride, but in the third period of administration

and in the first period following withdrawal there was a negative balance for sodium and chloride. A negative balance for potassium became apparent in the second period of administration of ACTH and in the third period of administration of cortisone.

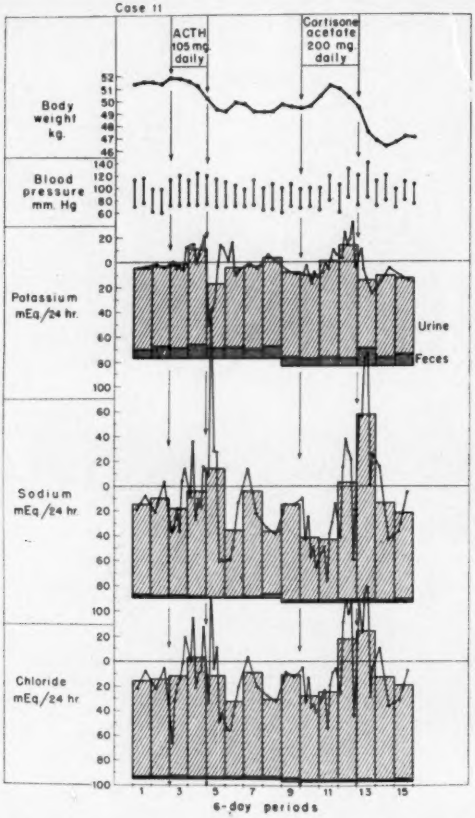

Fig. 15 (case 11).—Body weight, blood pressure and balance data for potassium, sodium and chloride.

In this case both ACTH and cortisone induced a negative nitrogen balance of considerable magnitude (fig. 16). Excretion of calcium and phosphorus in feces varied widely. There was a small but probably significant increase in urinary calcium and phosphorus during adminis-

tration of cortisone. Fecal calcium and phosphorus were rather markedly increased in period 13 after administration of the hormone was stopped.

CASE 6.—The patient in this case was a woman, 49 years of age, with severe chronic rheumatoid arthritis of three years' duration. In this case the metabolic effects of ACTH, 17-hydroxycorticosterone (compound F) and cortisone were

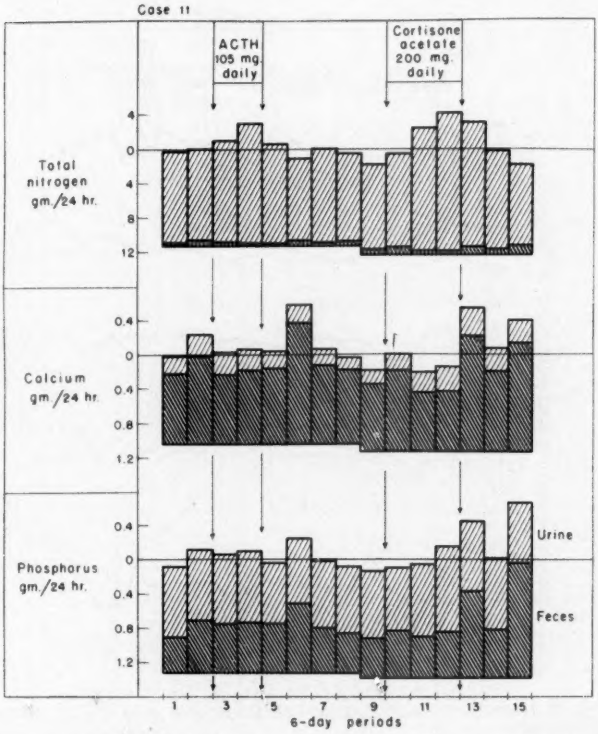

Fig. 16 (case 11).—Balance data for nitrogen, calcium and phosphorus.

studied. Changes in the concentration of the plasma electrolytes and in the balance for electrolytes can be seen by referring to figures 17 and 18, respectively. It will be noted that the alterations in the concentrations of the plasma electrolytes during and following administration of ACTH in a dose of 100 mg. daily were qualitatively similar to those previously observed in cases 11 and 14: There was a decrease in plasma sodium, a decrease of greater degree in plasma chlorides, which gave rise to a moderate increase in bicarbonate, and a decrease in potassium. In other words, a hypochloremic, hypopotassemic alkalosis developed which resembled that which is

seen in some cases of Cushing's syndrome except that the concentration of plasma sodium decreased. The balance data indicate that initially there was a marked retention of sodium and chloride but that in the second period of administration of ACTH (period 4), the balances for these ions become negative. On cessation of administration of ACTH, there was a marked loss of chloride and an even more marked loss of sodium (period 5). An apparent "rebound" occurred in period 6, so that there was a retention of sodium and chloride. ACTH induced a moderate

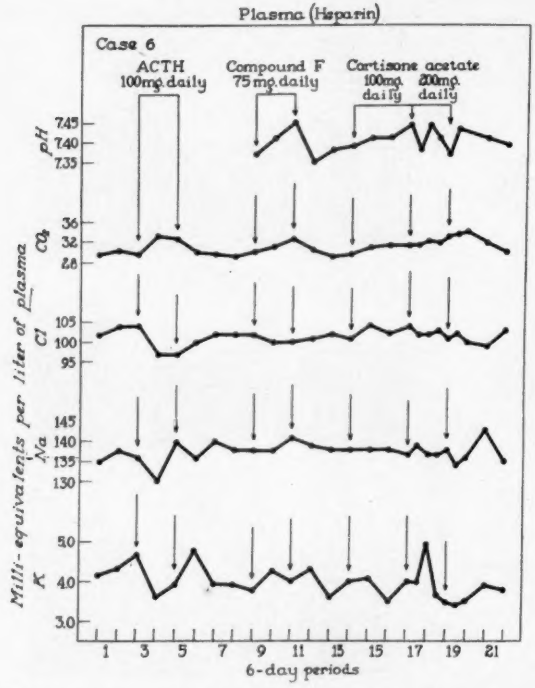

Fig. 17 (case 6).—Changes in plasma electrolyte pattern by ACTH, compound F and cortisone.

loss of potassium during the two periods of its administration; this was followed by retention of potassium in succeeding periods.

The administration of compound F in an average daily dose of 75 mg. for twelve days resulted in the development of an alkalosis of mild degree unaccompanied with significant change in the level of plasma sodium or potassium. In the first period of administration of compound F, the balances for sodium and chloride were slightly negative, but in the second period of its administration (that is, period 10) there was a retention of these ions. In the first period after use of compound F was stopped (period 11) there was a rebound with a loss of

chloride and a more marked loss of sodium. In the two succeeding periods (periods 12 and 13) there was retention of these ions of decreasing degree.

The administration of cortisone in a dose of 100 mg. daily for eighteen days followed by 200 mg. daily for twelve days resulted in the development of alkalosis which was qualitatively similar to that previously observed in cases

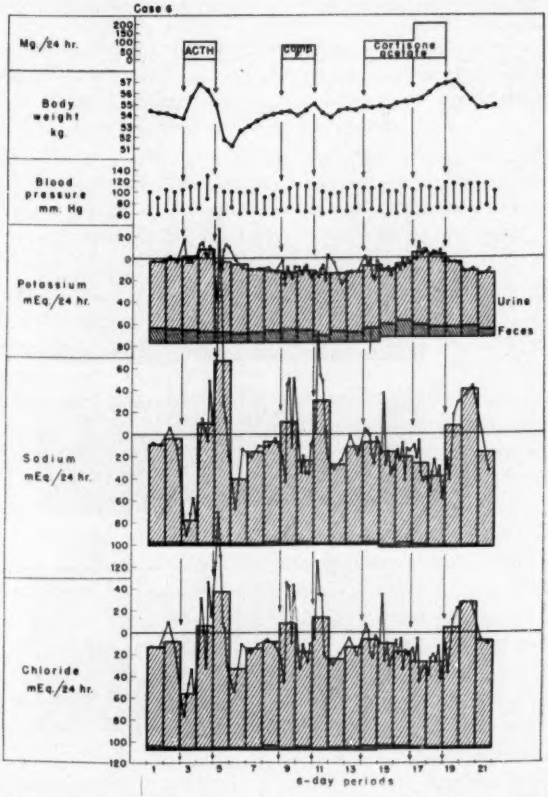

Fig. 18 (case 6).—Body weight, blood pressure and balances of potassium, sodium and chloride.

11 and 14. The balance data indicate that sodium and chloride were retained during administration of cortisone. However, in the two metabolic periods after administration of the hormone was stopped (that is, periods 19 and 20) the balances for these ions became negative. It is difficult to say whether the loss of sodium and chloride in these circumstances was due to continuing action of cortisone after cessation of its administration or to withdrawal of the hormone. However, as

already pointed out, other studies in which the balances for sodium and chloride became negative during administration of cortisone suggest that the former may be the case. This presumption is further supported by the observation in some cases that edema developed and subsequently decreased or disappeared while the hormone was still being administered.

Nitrogen balance became negative during administration of ACTH and cortisone; it did not become negative during administration of compound F (fig. 19).

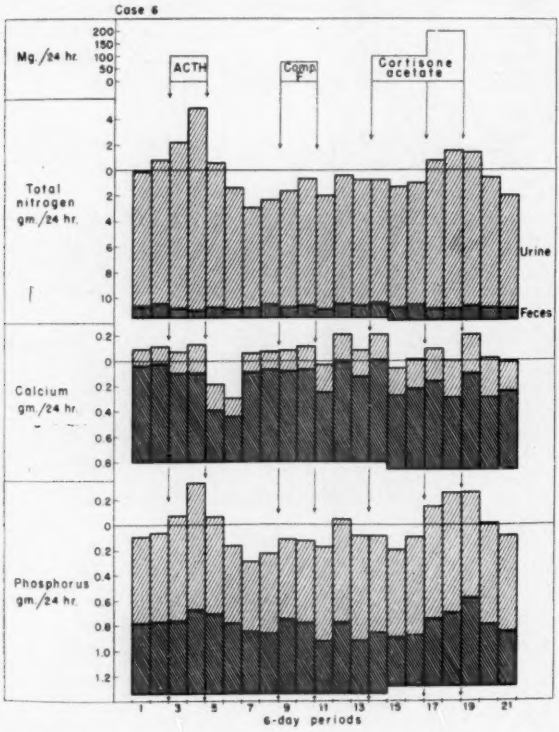

Fig. 19 (case 6).—Balances of nitrogen, calcium and phosphorus.

Urinary excretion of calcium and phosphorus increased during administration of ACTH and cortisone. In the two periods following the administration of ACTH (periods 5 and 6) there was a marked decrease of calcium in feces. The data for fecal phosphorus suggest an increase after administration of ACTH and cortisone, especially the later.

No significant changes were observed in serum calcium or phosphorus during the course of metabolic balance studies in cases 4. 5. 6. 11 and 14.

Summary of Changes Observed in Plasma Electrolytes and Metabolic Balances.—The complex character of some of the metabolic data, and the necessity for brevity make a brief summary of the most conspicuous changes desirable:

ACTH in a dose of 100 mg. daily for twelve days to 2 patients (cases 6 and 14) and 105 mg. daily for twelve days to 1 patient (case 11) induced a negative balance for nitrogen and potassium. During administration of the hormone there was initially a marked retention of sodium and chloride and then increased excretion of these ions. A hypochloremic, hypopotassemic alkalosis developed, accompanied with some lowering of the serum sodium.

Cortisone in a dose of 100 mg. daily for twelve to thirty days induced only minimal alterations, or no alteration at all, in the balances for nitrogen, calcium, phosphorus, sodium, potassium and chloride, and in the concentrations of electrolytes in the extracellular fluid as measured in blood plasma (cases 4 and 5).

Cortisone in a dose of 200 mg. daily for twelve to eighteen days regularly induced a negative balance for nitrogen and potassium. The effects on excretion of sodium and chloride were variable; the commonest was retention of these ions early in the period of administration of the hormone, followed later by increased excretion. At this dose level, a hypochloremic, hypopotassemic alkalosis developed, similar in all respects to that observed in some cases of Cushing's syndrome. The pattern of the blood electrolytes gradually reverted to normal after withdrawal of the hormone.

The metabolic effects of cortisone on a woman in whom artificial menopause had been induced four years previously (case 14) were not modified by the prior and simultaneous administration of 10 mg. of estrone daily. Later in this case the simultaneous administration of 25 mg. of testosterone propionate daily with the cortisone lessened the loss of nitrogen and minimized loss of potassium but did not prevent the development of a hypochloremic, hypopotassemic alkalosis.

The metabolic changes induced by the administration of compound F (900 mg. in twelve days) were not pronounced. Alkalosis of mild degree, associated with a slight decrease in plasma chlorides, developed.

There was no apparent correlation between the metabolic changes which resulted from the administration of ACTH, cortisone and compound F, and the favorable clinical effects of these hormones on the patients' rheumatoid arthritis.

Urinary Total Nitrogen.—The occurrence of negative nitrogen balance during administration, or immediately after withdrawal, of ACTH or cortisone has already been described (figs. 13, 16 and 19).

As a rule, augmentation of nitrogen excretion was slight or absent when the dose of cortisone was 100 mg. daily and pronounced when the dose was 200 mg. daily.

Uric Acid in Serum and Urine.—Excretion of uric acid was studied in cases 4, 5, 6, 11 and 14. In case 4 the average daily urinary excretion of uric acid during twelve days preceding administration of cortisone was 656 mg. During twelve days of administration of 100 mg. cortisone daily, the average was 698 mg. In case 5 the average daily excretion for eighteen days before cortisone was given was 807 mg., while during thirty days' administration of 100 mg. of cortisone daily the average was 850 mg. It is apparent that in these cases excretion of uric acid

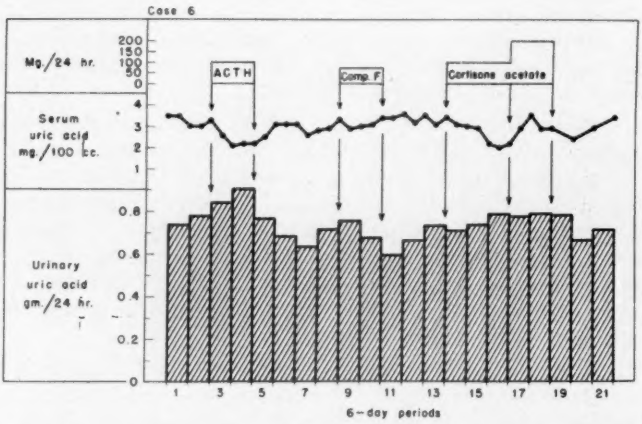

Fig. 20 (case 6).—Influence of ACTH, compound F and cortisone on uric acid in serum and urine.

was only slightly increased during the daily administration of 100 mg. of cortisone.

Data on uric acid in serum and urine in case 6 are shown in figure 20. ACTH in a dose of 100 mg. daily for twelve days induced a moderate increase in urinary excretion of uric acid and a decrease in blood uric acid. The changes observed during administration of compound F in an average dose of 75 mg. daily for twelve days were probably not significant. Likewise, the changes associated with the administration of cortisone in a dose of 100 mg. daily for eighteen days, followed by 200 mg. daily for twelve days were not pronounced. An apparently significant decrease in serum uric acid occurred during the first fifteen days of administration of cortisone, but was not maintained during continued administration, even at the higher dose level. There was a

slight rise in urinary excretion of uric acid which was probably significant in view of the decrease which occurred in the second six-day period after administration of the hormone was stopped (period 20).

In case 11, the administration of ACTH in a dose of 105 mg. daily for twelve days and cortisone in a dose of 200 mg. daily for eighteen days both resulted in significant increases in urinary uric acid (fig. 21). With ACTH there was a decrease in serum uric acid, and with cortisone there was also probably a significant decrease.

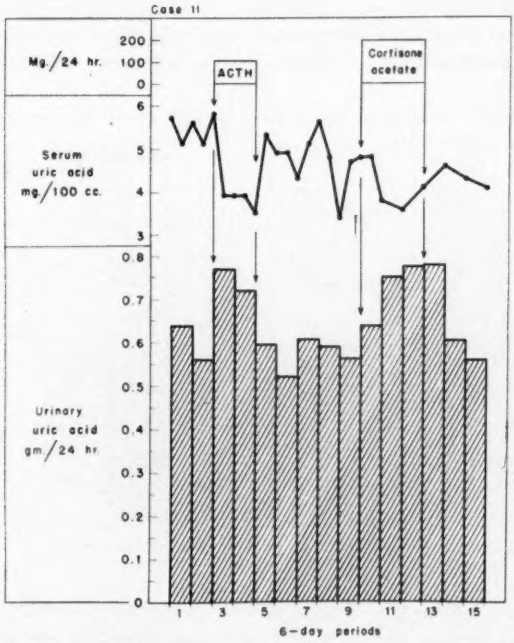

Fig. 21 (case 11).—Influence of ACTH and cortisone on uric acid in serum and urine.

In case 14 slight increases in urinary uric acid occurred during administration of ACTH, cortisone, cortisone plus estrone and cortisone plus testosterone (fig. 22). Changes in serum uric acid, which was initially at a low normal level, were variable and difficult to interpret.

In general, in the cases studied, significant decreases in serum uric acid under the influence of cortisone and ACTH occurred most commonly when the level was initially in the upper part of the normal range or above normal.

Creatine and Creatinine Nitrogen in Urine.—Urinary creatine nitrogen was increased during administration of ACTH and cortisone in cases 6, 11 and 14 (figs. 23, 24 and 25). The increases persisted for one or two six day periods after administration of the hormone was stopped. Prior and simultaneous administration of estrone did not prevent an increase in creatine nitrogen during and following administration of cortisone in case 14 (fig. 25). Simultaneous administration of testosterone propionate, on the other hand, initially caused a decrease in creatine nitrogen, and later apparently prevented in part the rise due to cortisone.

In case 4 the daily excretion of creatine nitrogen for twelve days prior to administration of cortisone averaged 164 mg. During admin-

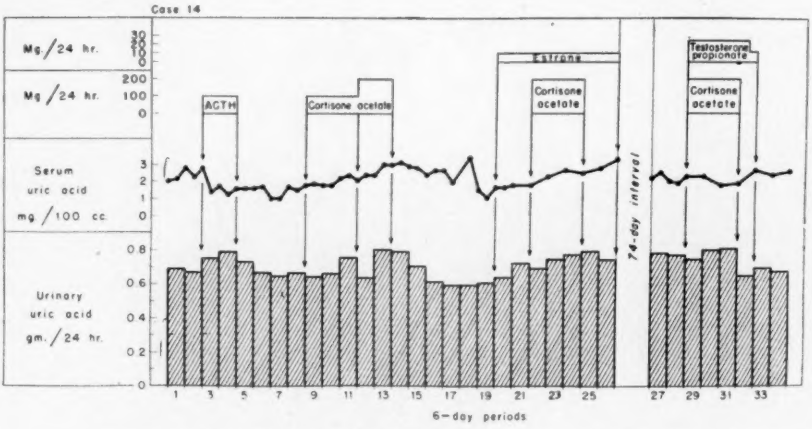

Fig. 22 (case 14).—Influence of ACTH, cortisone, cortisone plus estrone and cortisone plus testosterone propionate on uric acid in serum and urine.

istration of 100 mg. of cortisone daily for twelve days the daily excretion averaged 89 mg. The decrease in creatine nitrogen in this case, however, was probably not caused by the hormone, as the daily values, for reasons which are not clear, had been declining steadily even before administration of the hormone was begun.

In case 5 there was a slight increase in creatine nitrogen from an average of 82 mg. daily for eighteen days before administration of cortisone to 102 mg. daily for thirty days during administration of 100 mg. of cortisone daily.

With the possible exception of case 14, in no case were changes exhibited in urinary preformed creatinine nitrogen which could be

certainly attributed to ACTH or cortisone. In case 14 there was a signfiicant rise in preformed creatinine nitrogen during administration of cortisone plus testosterone propionate. In view of the previous absence of significant increase in preformed creatinine nitrogen during administration of ACTH or cortisone alone, it would appear that testosterone rather than cortisone was responsible.

Effects of Cortisone and ACTH on Carbohydrate Tolerance.—A number of experimental and clinical observations suggest that the administration of cortisone to man might cause impairment of tolerance for carbohydrate. Among these observations are the following: (1) Cor-

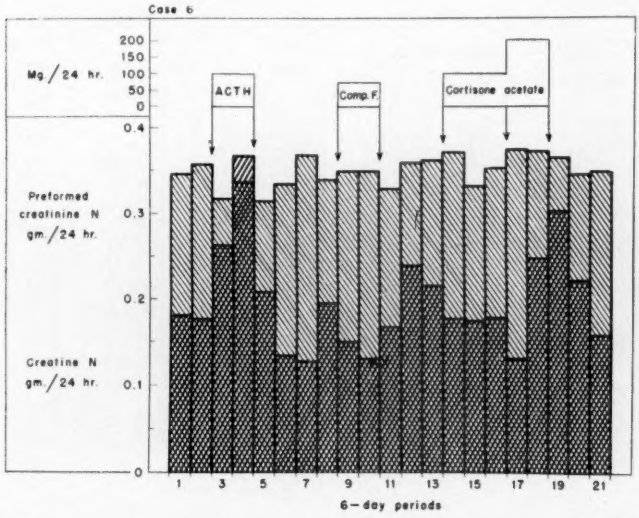

Fig. 23 (case 6).—Effect of ACTH, compound F and cortisone on urinary creatine and preformed creatinine, expressed as nitrogen. The double cross-hatched area represents creatine and the total height of each column represents creatinine except in period 4, when creatine exceeded creatinine.

tisone and ACTH are capable of inducing hyperglycemia and glycosuria in rats[55]; (2) impairment of carbohydrate tolerance is a frequent manifestation of Cushing's syndrome and is apparently directly related to overproduction of carbohydrate-active adrenal steroids in this con-

55. (*a*) Ingle, D. J.: The Production of Glycosuria in the Normal Rat by Means of 17-Hydroxy-11-Dehydrocorticosterone, Endocrinology **29**:649 (Oct.) 1941. (*b*) Ingle, D. J.; Li, C. H., and Evans, H. M.: The Effect of Adrenocorticotrophic Hormone on the Urinary Excretion of Sodium, Chloride, Potassium, Nitrogen and Glucose in Normal Rats. ibid. **39**:32 (July) 1946.

dition [4c]; (3) cortisone lessens sensitivity to insulin in patients having Addison's disease,[4e] and greatly intensifies the diabetes of patients who have coexisting Addison's disease and diabetes mellitus,[56] and, if the observations in the case reported by Boland and Headley [6b] are generally true, augments the requirement for insulin of diabetic patients receiving cortisone.

The doses of cortisone administered to patients who were subjects of this study (usually about 100 mg. daily) were relatively small compared to those with which Ingle [55a] induced frank diabetes in animals. He administered 5 to 10 mg. daily to rats, obviously relatively large

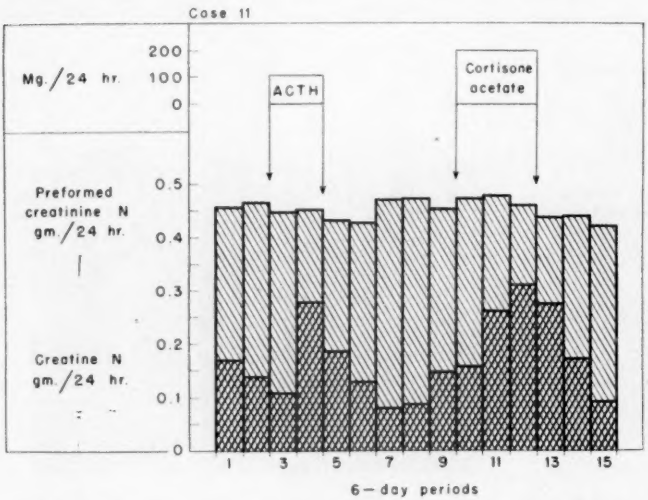

Fig. 24 (case 11).—Effect of ACTH and cortisone on urinary creatine and preformed creatinine, expressed as nitrogen. Creatine and creatinine are designated in the same manner as in figure 23.

doses on the basis of body weight. Assuming that there is some validity in this implied analogy between the carbohydrate metabolism of man and rat, it would be anticipated that if any impairment of carbohydrate tolerance occurred in the subjects of our study it would be slight.

This reasoning proved to be correct. Although slight inconstant increases in the fasting blood sugar were observed in some cases during administration of cortisone or ACTH, the values never exceeded the normal range. For the entire group of patients who received cortisone

56. Footnotes 4 *c*, 4 *e* and 5 *a*.

and for whom the concentration of blood sugar was determined, there were no significant changes in mean values during or after administration of the hormone as compared to the mean value before administration of the hormone[57] (table 3). More than traces of sugar in the urine were not observed in any case.

Glucose tolerance tests, which are somewhat more sensitive than determinations of the fasting blood sugar in the detection of impairment of carbohydrate tolerance, were performed in a small group of cases.[58] None of the patients was known to have diabetes. Most of the tests were performed during administration of cortisone, but a few were

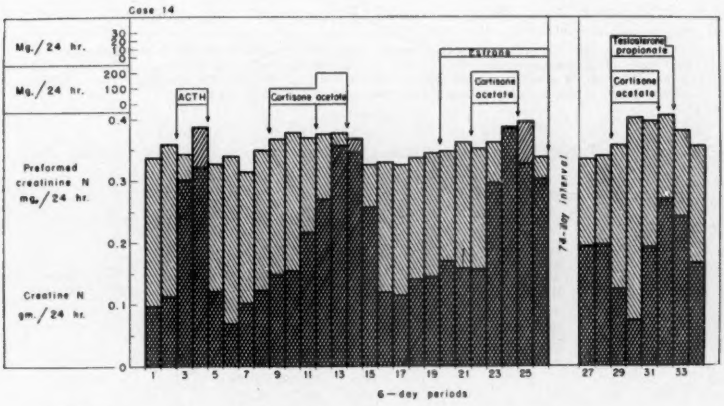

Fig. 25 (case 14).—Effect of ACTH, cortisone, cortisone plus estrone and cortisone plus testosterone propionate on urinary creatine and preformed creatinine, expressed as nitrogen. Creatine and creatinine are designated in the same manner as in figure 23. In periods 4, 14 and 25 creatine exceeded creatinine.

57. The available data for fasting blood sugar, serum albumin, serum globulin and zinc sulfate turbidity were treated as follows: The observed values before administration of cortisone was instituted, during the time that the hormone was being administered and after administration was stopped were contrasted. The pertinent differences were calculated for each subject, that is, the difference between the values obtained before and during administration, and the difference between the values obtained before and after administration. The mean of those differences, together with its standard error, was then calculated from the individual differences.

The Division of Biometry and Medical Statistics assisted in making the statistical calculations included in this article.

58. Glucose tolerance tests were not performed during administration of ACTH. However, as pointed out earlier, Conn and his associates have demonstrated the diabetogenic potentialities of ACTH in the human being.[11]

performed before and after its administration as well. Although the data do not permit conclusions concerning the incidence of impairment of carbohydrate tolerance by cortisone, they do indicate that measurable impairment can occur in some cases (table 4). Abnormal glucose tolerance curves, presumably caused by cortisone, were observed in

TABLE 3.—*Changes in Level of the Fasting Blood Sugar During and After Administration of Cortisone*

Mean Value Before Administration, Mg. per 100 Cc.	During Administration		After Administration	
	Cases	Mean Difference, Mg. per 100 Cc.	Cases	Mean Difference, Mg. per 100 Cc.
81.9	18	−1.5 ± 1.7*	16	−2.2 ± 2.0*

* A minus sign (−) indicates a decrease as compared with the level before administration of cortisone. The value following the ± sign is the standard error of the mean difference. The mean difference is considered statistically significant if it is twice its standard error. Therefore, neither of the changes tabulated is considered statistically significant.

TABLE 4.—*Results of Glucose Tolerance Tests Performed Before, During and After Administration of Cortisone Acetate*

Case	Family History of Diabetes	Before Administration, Blood Sugar, Mg. per 100 Cc.	During Administration			After Administration	
			Days Given (Consecutive)	Average Dose, Mg.	Blood Sugar, Mg. per 100 Cc.	Days Since Last Dose	Blood Sugar, Mg. per 100 Cc.
1	No		169	90	88, 135, 185, 85		
9	No	90, 195, 230*	15	110	88, 173, 149*		
10	No	83, 149, 153*	13	154	83, 120, 153*		
11	Yes	78, 125, 103, 86	7	129	86, 149, 144, 86		
			16	200	98, 142, 153, 81	18	74, 102, 88, 60
12	No		7	129	88, 163, 86, 74		
			56	105	83, 163, 149, 53	128	90, 135, 63, 64
13	No	83, 135, 63, 66	9	122	85, 163, 85, 56		
14	Yes		15†	200	86, 103, 147, 103		
15	No		60	131	86, 113, 107, 72		
23	No	88, 164, 132, 87	11	118	78, 125, 115, 74		
			36	106	78, 115, 93, 81		
			12	100	86, 120, 111, 100		
25	No		11	127	78, 142, 100, 60		
26	No					20	78, 115, 86, 60
32	No		96	112	80, 141, 54, 78		
			162	107	78, 128, 74, 70		

* Exton and Rose's glucose tolerance test. Other tests were of "standard" type, in which 1 Gm. of glucose per kilogram of body weight was given and the blood sugar determined at 0, one-half or one, two and three hours.

† This patient received 10 mg. of estrone daily in addition to cortisone.

cases 1, 11, 12 and 14. In 3 (cases 1, 12 and 14) of these 4 cases, the tests had not been performed prior to administration of the hormone. In case 12, a normal curve on administration of cortisone for only seven days adds significance to the abnormal curve which was found on administration of the hormone for fifty-six days. Furthermore, glucose tolerance in this case had reverted to normal one hundred and twenty-

eight days after administration of the hormone was stopped. In cases 1 and 14 the lack of control data necessitates some caution in interpretation of the abnormal curves which were found during administration of cortisone. An aunt of the patient in case 11 had diabetes. The glucose tolerance in this case was normal both before administration and after withdrawal of cortisone, but it was abnormal during two

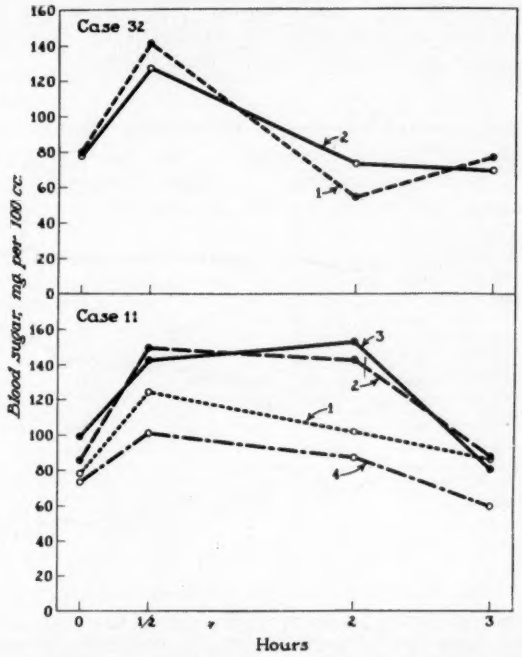

Fig. 26.—*Upper* (case 32).—(*1*) Normal glucose tolerance curve after administration of an average of 112 mg. of cortisone daily for ninety-six days. (*2*) Normal curve after administration of an average of 107 mg. of cortisone daily for one hundred and sixty days. *Lower* (case 11).—(*1*) Normal curve before cortisone. (*2*) Abnormal curve after an average of 129 mg. of cortisone for seven days. (*3*) Abnormal curve after 200 mg. of cortisone daily for sixteen days. (*4*) Normal curve eighteen days after last dose of cortisone.

different periods of administration of the hormone. In the first of these, the patient had received only 900 mg. of cortisone in seven days.

In contrast to the cases in which administration of cortisone resulted in evidence of impairment of carbohydrate tolerance, it will be seen by reference to table 4 that in other cases, particularly cases 15, 23 and

32, there was no measurable alteration of tolerance after administration of the hormone for protracted periods. Glucose tolerance curves in case 32, in which no abnormality developed on prolonged administration of cortisone, and in case 11, in which impairment of tolerance did develop, are shown in figure 26.

It is apparent, then, that cortisone in the doses which were employed usually did not produce conspicuous alterations in carbohydrate tolerance. It is equally apparent that more striking changes in carbohydrate tolerance might be expected if the hormone were administered to persons having latent or frank diabetes.

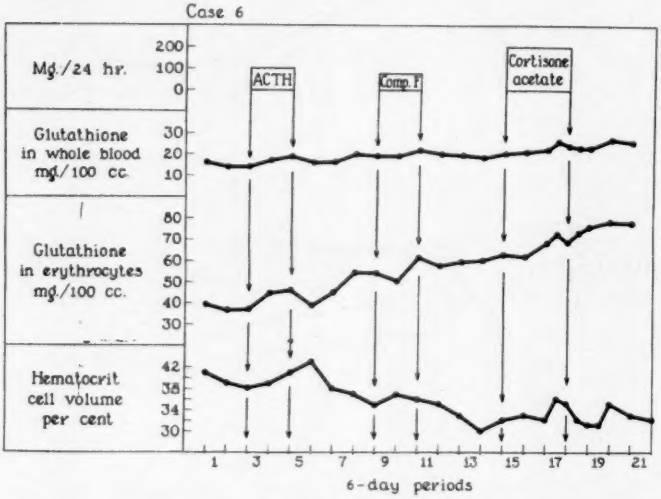

Fig. 27 (case 6).—Glutathione in whole blood and in erythrocytes. As the hematocrit reading (cell volume per hundred cubic centimeters) decreased during the course of the study, the concentration of glutathione in erythrocytes increased in compensatory fashion.

Glutathione in the Blood.—Determinations of glutathione in whole blood and erythrocytes were made at intervals of six days throughout the course of metabolic studies in cases 6, 11 and 14. The data in case 6, which are similar to those obtained in cases 11 and 14, are shown in figure 27. During the entire period of observation there was in each case a progressive drop in concentration of hemoglobin, erythrocyte count and hematocrit reading, associated with a gradual rise in the concentration of glutathione in erythrocytes. The quantity of gluta-

thione in whole blood changed little. During administration of hormones there was no consistent pattern of change in the 3 cases. For example, during administration of ACTH in cases 6 and 11, there was a slight increase in glutathione in whole blood and erythrocytes, while in case 14 there was a slight decrease. During administration of cortisone in all 3 cases there were slight increases in glutathione in whole blood and erythrocytes. Adequate data are not available for a significant correlation of changes in concentration of glutathione in blood and erythrocytes with alterations in glucose tolerance, although abnormal glucose tolerance curves were observed in cases 11 and 14 during administration of cortisone.

Serum Albumin, Globulin and Zinc Sulfate Turbidity.—As previously noted by Hench and his associates,[2a] elevated values of serum globulin and depressed levels of serum albumin usually changed toward normal during administration of cortisone. Indeed, the same direction

TABLE 5.—*Changes in Serum Albumin, Globulin and Zinc Sulfate Turbidity During Administration and After Withdrawal of Cortisone*

	Mean Value Before Administration	During Administration		After Withdrawal	
		Cases	Mean Differences*	Cases	Mean Difference*
Albumin	4.0†	26	+0.18 ±0.07†	28	+0.22 ±0.9†
Globulin	3.1†	26	−0.67 ±0.06†	28	−0.72 ±0.10†
Zinc sulfate turbidity	20‡	20	−7.0 ±1.8‡	16	−0.1 ±2.8‡

* A plus sign (+) indicates an increase as compared with the level before administration of cortisone, a minus sign (−) indicates a decrease. The value following the ± sign is the standard error of the mean difference. The mean difference is considered statistically significant if it is twice its standard error. Therefore, all of the changes tabulated above are considered statistically significant.

† Gm. per 100 cc. of serum.

‡ Units.

of change in the serum proteins was observed in some cases in which the initial levels were within normal limits. A considerable mass of data on serum albumin and globulin in all the cases in which they were studied is summarized in table 5. It will be noted that the mean difference in the values of serum albumin obtained during administration of cortisone, as contrasted to the values obtained prior to administration of the hormone was slightly positive and that the increase was maintained when administration of the hormone was discontinued. The mean differences in the values of serum globulin were greater and in the opposite direction. Stated simply, the effect of cortisone was to increase serum albumin slightly and to decrease serum globulin to a greater degree.

Zinc sulfate turbidity, which presumably is a rough measure of serum gamma globulins, decreased significantly during administration of cortisone. The decrease was maintained after administration of the hormone was stopped (table 5).

Other Blood Chemical Studies.—Periodic determinations of the serum alkaline phosphatase in cases 1, 6, 11 and 14 revealed no consistent pattern of change during administration of cortisone or ACTH. All values observed were within normal limits. Likewise, the blood urea did not vary significantly in any of the numerous cases in which periodic determinations were made. The total blood cholesterol increased during administration of cortisone in 4 cases (cases 1, 2, 5 and 9) out of 6 in which determinations were made, but the data are not adequate to establish the validity of the observation.

Basal Metabolism and Respiratory Quotient.—In case 6 the average value of the basal metabolism on 5 determinations prior to administration of ACTH was +22 per cent. There was no clinical evidence of thyrotoxicosis, and the thyroid gland was of normal size and consistency. During administration of ACTH the basal metabolic rate was 0 per cent on two occasions. Twenty-four days after withdrawal of ACTH, the rate had gradually increased to + 13 per cent. During administration of compound F the mean value was + 5 per cent. Following this, during 15 days when no hormones were being administered, the mean of three determinations was +16 per cent. Then, during administration of cortisone, it gradually fell to a low value of —3 per cent and remained in this range (—3 to —9 per cent) during twelve days of observation following cessation of administration of cortisone.

It was felt at the time that the hypermetabolism before and in the intervals between administration of hormones in this case was probably related to the patient's rheumatoid arthritis rather than to hyperthyroidism and that the apparently significant decrease in basal metabolism during administration of hormones was related to improvement in the rheumatic state. The mechanism involved, however, merits further study.

In cases 11 and 14, there was no consistent pattern of change in the basal metabolism in relation to cortisone or ACTH.

Consistent changes in the respiratory quotient attributable to cortisone or ACTH were not observed in cases 6, 11 or 14.

EFFECTS ON LEUKOCYTES

Cortisone.—Data on changes in leukocytes under the influence of cortisone are to be presented in detail by Reynolds and Heck in another publication. Suffice it to say here that statistical analysis of data obtained in the entire group of cases has revealed a small but significant increase in the total number of circulating leukocytes during protracted administration of cortisone and no significant change in the number of lymphocytes or eosinophils either during or after administration of

the hormone. The results are derived from prolonged administration of cortisone and are not necessarily a contradiction of the observation of others of a significant decrease in lymphocytes and eosinophils during short periods of administration of the hormone. Moreover, the results cited do not deny the occurrence of significant decreases in lymphocytes and eosinophils in certain individual cases. Indeed, such decreases were actually observed.

ACTH.—In cases 6, 11 and 14 complete, or almost complete, disappearance of circulating eosinophils occurred on the fourth to the sixth day of administration of ACTH, which persisted as long as the hormone was given. Counts made on the fourth day after withdrawal of ACTH disclosed a return of eosinophils to higher than control levels. Subsequently they decreased to approximately the control levels.

EVIDENCES OF DEPRESSION OF ADRENAL CORTEX FUNCTION BY CORTISONE

Administration of cortisone or other adrenal hormones which have similar physiologic activity to animals causes atrophy of the adrenal cortices. An analogous situation occurs in patients who have functioning tumors of the cortex of one adrenal gland, particularly when the tumor induces Cushing's syndrome. When the source of excess adrenal hormones, presumably cortisone-like, is removed by surgical excision, a temporary state of adrenal cortex insufficiency is created, because of atrophy of the contralateral adrenal gland. In both animal and man, the adrenal atrophy is presumably attributable to suppression of the adrenocorticotropic function of the anterior lobe of the pituitary body by an excess of adrenal hormones in the body.

On the basis of these observations, it might be anticipated that cortisone would cause depression of function of the adrenal cortices in the human being and that such depression might be apparent for an interval following cessation of administration of the hormone, particularly if the hormone had been administered in large doses for a prolonged period. Actually, three lines of evidence suggestive of adrenal depression by cortisone, no one of them conclusive, were obtained, as follows:

Asthenia Following Withdrawal of Cortisone.—As already pointed out, several patients complained of weakness of varying degree which began a few days after administration of cortisone was stopped. Asthenia was particularly pronounced in cases 3, 5 and 14. The duration of asthenia varied from a few days to several weeks. There was no consistent relation of asthenia to recurrence of articular disease after withdrawal of cortisone. The asthenia was not associated with significant abnormalities of the concentrations of the blood electrolytes or with hypoglycemia. Evidence of severe adrenal cortex insufficiency was not

observed. Hiccups or nausea did not occur. Cutaneous pigmentation was not observed. Specific treatment for adrenal cortex insufficiency was not necessary in any case, and was not administered.

Depressed Excretion of 17-Ketosteroids During Administration and After Withdrawal of Cortisone.—A prompt decrease in urinary excretion of 17-ketosteroids soon after administration of cortisone was started, and persistently low levels following cessation of prolonged administration of the hormone (figs. 5 and 6) would seem to indicate depression of at least some functions of the adrenal cortex.

TABLE 6.—*Changes in Eosinophil Count in Response to 25 Mg. of ACTH Before and During Administration and After Withdrawal of Cortisone*

	Before Administration, Eosinophils per Cu. Mm.			During Administration					After Withdrawal					
				Time Given, Days (Consecutive)	Average Daily Dose, Mg.	Eosinophils per Cu. Mm.			Time Given, Days (Consecutive)	Average Daily Dose, Mg.	Days Since Last Dose	Eosinophils per Cu. Mm.		
Case	8 A.M.	12 M.	% Change			8 A.M.	12 M.	% Change				8 a. m.	12 M.	% Change
3	...	..	....	..	...	...	...	....	73	100	33	556	408	—28
	...	..	....	...	...	...	...	....			86	425	145	—66
6	148	35	—76	..	...	...	...	....	..	...	..	...	...	....
12	...	..	....	..	...	...	...	....	57	100	8	66	65	—2
	...	..	....	...	...	...	...	....			129	258	35	—86
14	62	6	—90	18	200	9	3	—66	18	200	18	26	9	—65
	38	9	—76	..	...	...	...	....	..	...	..	...	...	....
16	...	..	....	145	124	121	84	—31	161	117	10	153	18	—88
19	...	..	....	...	...	...	...	....	66	103	9	376	334	—11
21	...	..	....	35	100	210	160	—24	56	100	11	121	115	—5
22	616	122	—80	20	100	229	257	+12	32	100	10	151	73	—52
	...	..	....	32	100	357	341	—4	32	100	56	473	163	—66
23	163	31	—80	20	100	223	103	—54	30	100	19	73	0	—100
	...	..	....	29	100	160	21	—87*	..	...	..	...	...	....
	...	..	....	15	100	125	156	+25	..	...	..	...	...	....
32	...	..	....	...	...	...	...	....	166	107	6	138	68	—5
	...	..	....	...	...	...	...	....	166	107	27	163	76	—53

* Urticarial reaction after ACTH.

Response of the Adrenal Cortices to ACTH.—A series of tests of adrenal response to a single 25 mg. dose of ACTH was performed, as described by Thorn and his associates.[12] Response was measured by change in the number of circulating eosinophils four hours after the injection of ACTH.[59] The results are summarized in table 6. If a normal response is defined (on the basis of the experience of Thorn and his associates[12] and that of Balfour)[60] as a 62 per cent or greater decrease of circulating eosinophils, it will be seen that all of the 5 patients tested responded normally to ACTH prior to administration of cortisone. This is in agreement with the observation of Thorn and

59. Changes in the urinary uric acid–creatinine ratio were also determined. In agreement with the experience of others, it was found that there was much greater variability in response to ACTH in terms of change in this ratio than in terms of decrease in the number of circulating eosinophils.

60. Balfour, W. M.: Unpublished data.

his associates [9] that most patients with rheumatoid arthritis studied so far have a normal eosinophilic response to ACTH. During administration of cortisone, response to ACTH was normal in 2 tests and abnormal in 6. The 2 "normal" responses are open to question because of the extremely low initial eosinophil count in 1 (case 14) and the occurrence of a moderately severe urticarial reaction to ACTH in the other (case 23). At variable intervals after administration of cortisone was stopped, the response to ACTH was variable, being normal six times and abnormal six times. In cases 3, 12 and 22 the response changed from abnormal to normal as the interval after the last dose of cortisone increased. Thus, if it can be assumed that cortisone does not induce a change in the eosinophils themselves which makes them less responsive to adrenal cortex steroids, the over-all results are strongly suggestive of a decrease in response of the adrenal cortices to ACTH as a result of administration of cortisone.

The three lines of evidence suggesting depression of adrenal cortex function which have been cited, taken together, create a strong presumption that such functional depression may actually occur, particularly in the light of evidence from animal experiments. Larger doses of cortisone might be expected to produce more significant depression. It is not likely to present a serious practical problem, however, except under conditions of stress, when adrenal function might conceivably be inadequate.

COMMENT

It seems apparent from the clinical and metabolic studies which have been presented, as well as from the observations of others on animals and human beings, that ACTH and cortisone are powerful hormonal substances which are capable of influencing many physiologic processes. Brief comment will be made on some of the most conspicuous physiologic alterations observed in our group of cases.

Features of Cushing's Syndrome.—Many of the manifestations of Cushing's syndrome were induced by protracted administration of cortisone. These included some features (mild hirsutism, acne, keratosis pilaris, mental depression, amenorrhea, edema and alkalosis) which formerly were not thought by many (including some of us) to be caused by excessive amounts of cortisone-like steroids. In addition, certain other features of the syndrome which have been presumed in the past to be related to overproduction of cortisone-like steroids were observed. These included cutaneous striae, muscular weakness, impairment of carbohydrate tolerance, negative nitrogen balance and increased urinary excretion of corticosteroids. On the grounds that most of the classic features of Cushing's syndrome were observed within the group of

patients who received relatively large amounts of cortisone, one can reasonably speculate that the entire syndrome as seen in association with hyperfunctioning lesions of the adrenal cortices may be the result of overproduction of cortisone-like steroid hormones. Final proof of this proposition is, of course, still lacking.

Urinary Steroids.—If proof of the foregoing speculation concerning the pathogenesis of spontaneous Cushing's syndrome is forthcoming in the future, there is already good reason to suspect that 17-hydroxycorticosterone (compound F) rather than cortisone may be the hormone responsible for the production of the syndrome. The principal lines of evidence in support of this supposition are: (1) the known similarity in the physiologic effects of compound F and cortisone; (2) the isolation of relatively large amounts of compound F from the urine in cases 6, 11, 14 and 17 when the adrenal cortices were stimulated with ACTH, and (3) a similar finding in the case of a boy with spontaneous Cushing's syndrome associated with hyperplasia of the adrenal cortices.[61] In any event, the evidence is suggestive that stimulation of the adrenal cortex of human beings by ACTH provokes the secretion of compound F rather than cortisone.

Excretion of corticosteroids was usually augmented during administration of cortisone. The same observation was previously made in the case of 2 female patients having Addison's disease who received cortisone.[4e] In both cases a small amount of unchanged cortisone was isolated from the urine. The amount of corticosteroids measured in the urine by chemical means was small relative to the amount of cortisone administered. Not more than 5 per cent, and often much less, of the cortisone administered could be found in the corticosteroid fraction.

The urinary excretion of 17-ketosteroids, on the other hand, decreased promptly when administration of cortisone was begun. In some cases the decrease persisted long after administration of the hormone was stopped. This evidence points to depression of at least some functions of the adrenal cortices by cortisone. The latter presumption is not invalidated by the observations that administered cortisone caused an increase in urinary excretion of 17-ketosteroids in female patients having Addison's disease and that large doses of cortisone in time caused an increase, rather than a decrease, in the 17-ketosteroids in the urine of patients in this series, all of whom presumably had intact adrenal glands. All these observations are consistent with the hypothesis, therefore, that 17-ketosteroids which appear in the urine during administration of cortisone are derived

61. Mason, H. L., and Sprague, R. G.: Isolation of 17-Hydroxycorticosterone from the Urine in a Case of Cushing's Syndrome Associated with Severe Diabetes Mellitus, J. Biol. Chem. **175**:451 (Aug.) 1948.

largely from administered cortisone. If this is true, the 17-ketosteroids excreted in our cases represented a maximum of only 5 to 10 per cent of the cortisone administered. After a long period of administration of the hormone, the amount which appeared in the urine as 17-ketosteroids was even less (only 2 to 3 per cent of the amount given).

Nitrogen.—The loss of nitrogen which was observed during or after administration of large doses of cortisone (200 mg. daily) and ACTH (100 mg. daily) is indicative of an increase in the catabolism of protein under the influence of the hormones. Likewise, the retention of nitrogen which was observed when the effects of the administered hormones had waned is indicative of an antecedent loss of protein. The marked creatinuria which was observed when the hormones were administered probably has a similar general significance, for creatinuria commonly occurs in association with the metabolism of muscular tissue. The increased excretion of uric acid, sometimes unassociated with significant change in the level of uric acid in the blood serum, is probably consistent with the same general interpretation.

Electrolyte Effects.—As pointed out earlier, the details of the effects of cortisone and ACTH on electrolyte metabolism need further investigation. From a practical standpoint, however, it is apparent that these hormones may cause significant retention of salt and water, sometimes with the production of edema. That this effect may not persist, however, is indicated by the development of negative balances for sodium and chloride during continued administration of cortisone and the observation that edema may decrease or disappear in some patients while the hormone is being given.

Loss of potassium from the body under the influence of cortisone and ACTH is well documented. The experimental data indicate that in some instances a sufficient amount of potassium appears in the urine during administration of the hormones to indicate that it may be derived from both the extracellular and the intracellular fluid.

Carbohydrate Metabolism.—Changes in carbohydrate tolerance as measured by determinations of the fasting blood sugar and by glucose tolerance tests were not pronounced. There is little reason to doubt, however, on the basis of Ingle's observations in rats,[55] that the employment of larger doses of cortisone and ACTH or the presence of antecedent mild impairment of islet function would give rise to more conspicuous changes. In this connection, it should be pointed out that glycosuria observed during administration of cortisone or ACTH is not necessarily solely diabetic in character, for in the case of ACTH, at least, there is evidence which indicates that it depresses the renal threshold for glucose.[11]

Functional Depression of the Adrenal Cortices.—The evidence cited herein is strongly suggestive of depression of some functions of the adrenal cortices by cortisone. The evidence consists of asthenia after withdrawal of cortisone, depression of the urinary excretion of 17-ketosteroids during administration and after withdrawal of cortisone and impairment of the eosinophilic response to ACTH. It seems probable that more conclusive evidence of depression of the adrenal cortices might become apparent if even larger doses of cortisone than were employed in this study were administered for prolonged periods. From a practical standpoint, the possibility should be borne in mind that function of the adrenal cortex might be inadequate under conditions of stress, such as fever or trauma, in a patient who had recently received cortisone.

General Comment. — Our observations are presented with the thought that they may provide some guidance for further physiologic studies in human subjects and also because they have obvious bearing on the eventual therapeutic use of these hormones in disease. Certainly the data which have been presented do not constitute an exhaustive investigation of the physiologic potentialities of these materials. Nevertheless, their far reaching effects when given in sufficient dosage have been demonstrated. While it is not our primary purpose to comment on questions of therapeutics, the inference of these observations is obvious; the profound physiologic effects of cortisone and ACTH will necessarily call for careful consideration and a balance in their therapeutic use between what is most desirable from the standpoint of the disease in question, on the one hand, and what is best from the standpoint of the overall welfare of the patient, on the other.

It is perhaps redundant to point out that the multifarious physiologic alterations induced by these hormones are more appropriately regarded as "effects" than as "side effects" or "toxic reactions." It is evident that the latter two terms, and others like them, depend on a clinically restricted appreciation of the biologic significance of these substances.

It is clearly apparent, furthermore, that these materials provide an introduction to a better understanding of several hitherto obscure clinical conditions. It is hoped that the data presented in this paper will be of help to others in the future application of cortisone and ACTH as tools for the investigation of such conditions.

SUMMARY

Clinical and metabolic studies of 33 patients who received cortisone or ACTH or both for varying periods are reported. One of the patients also received compound F. In most of the cases the hormones were administered for the primary purpose of studying their effects on rheu-

matoid arthritis, rheumatoid spondylitis, acute rheumatic fever, disseminated lupus erythematosus or psoriasis with psoriatic arthritis. In other cases they were administered to patients with rheumatoid arthritis for the purpose of making more precise observations of their metabolic effects. Our studies were concerned chiefly with the effects of fairly prolonged administration of the hormones. While more observations were made of the effects of cortisone than of ACTH, it was apparent, as expected, that the two hormones had many effects in common.

Although the observations are reported without special reference to questions of the practical use of these substances in the investigation and treatment of certain disease states, it is recognized that some of the effects which were observed have direct bearing on the possible use of the hormones as therapeutic agents. The clinical details of their effects on the condition for which they were administered are to be reported by Hench and his associates. The data reported confirm what has long been known or suspected, namely, that cortisone and ACTH are powerful hormonal agents which influence a variety of physiologic processes.

The capacity of cortisone when administered in sufficiently high dosage for a protracted period to induce most of the clinical and metabolic features of Cushing's syndrome was demonstrated. The features so induced in varying combinations included rounding of the facial contour, hirsutism, acne, keratosis pilaris, muscular weakness, edema, amenorrhea, cutaneous striae, mental depression, impairment of carbohydrate tolerance, negative nitrogen balance, increased excretion of corticosteroids in the urine and hypochloremic hypopotassemic alkalosis. Significant hypertension was observed in only 1 case, in which the patient probably had antecedent parenchymatous renal disease.

In addition, metabolic studies disclosed that cortisone and ACTH increased excretion of creatine and uric acid in the urine and gave rise to a negative balance for potassium in some cases. Their effects on balances of sodium and chloride were variable, the usual pattern being retention of salt early in the period of administration of the hormone followed by increased excretion when administration was prolonged. In some cases there were slight increases in urinary excretion of calcium and phosphorus. Fecal phosphorus, and possibly calcium, increased significantly in at least 1 case. Compound F in an average dose of 75 mg. daily for twelve days did not have pronounced metabolic effects.

Studies of the urinary steroids provided evidence that the adrenal cortex of man when stimulated by ACTH secretes 17-hydroxycorticosterone (compound F) rather than cortisone. Evidence was obtained that a small percentage of administered cortisone is excreted in the urine as 17-ketosteroids and corticosteroids. Small amounts of unchanged cortisone were present in the corticosteroid fraction.

Evidence was obtained that cortisone is capable of depressing the function of the adrenal cortices in man, as it does in the rat.

In this study the over-all effects of protracted administration of cortisone on leukocytes were not pronounced.

The suggestion is made that such terms as "side effects" or "toxic reactions" not be applied to physiologic alterations induced by cortisone and ACTH which do not have favorable therapeutic implications, for these designations do not indicate a broad appreciation of the biologic significance of these important substances.

BACTERIAL MENINGITIS

Results of Treatment in Seventeen Cases with a New Sulfonamide (Gantrisin®)

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IN A PREVIOUS communication,[1] our clinical experience with gantrisin,® a new sulfonamide with the formula 3,4-dimethyl-5-sulfanilamido-isoxazole, was reported. Its use as the sole bacteriostatic agent in 53 patients with various infections and in combination with antibiotics in 38 others was reported, the entire series including practically all the conditions for which sulfonamides are currently used. The results on the whole were satisfactory. In the series of 91 patients reactions of some type occurred in 9, anorexia in 1, nausea in 5, vomiting in 2, headache in 1, a generalized eruption in 1 and crystalluria in 2. No hematuria or renal blocking was noted. More than 100 patients have been treated since that report with approximately the same results.

Since many of the conditions for which sulfonamides are commonly prescribed are self limiting, we felt that a separate and somewhat more detailed report of our results in bacterial meningitis—a disease in which a large percentage of the cases do not clear up spontaneously—might be of interest.

MENINGOCOCCIC MENINGITIS

Gantrisin® was the only bacteriostatic agent used in the treatment of 10 patients suffering from meningococcic meningitis (table 1). For 9 of this group the diagnosis was made by recovering meningococci from the spinal fluid or the blood, or both. In the other patient the spinal fluid and the blood were sterile on culture, but 750 cells, mostly polymorphonuclear leukocytes, were found in the spinal fluid and the typical petechial hemorrhages of meningococcemia were present; hence, she was included in the series. As can be seen from the summary, treatment was begun in this group within three days of the onset, so the patients

Gantrisin was formerly known as NU 445.

From the Department of Medicine, Northwestern University Medical School.

1. Rhoads, P. S.; Svec, F. A., and Rohr, J. H.: A Clinical Evaluation of a New Sulfonamide—Gantrisan, Quart. Bull., Northwestern Univ. M. School **23**:104-111, 1949.

represent a selected group to that extent. They appeared to have attacks of average severity. The total dose for the first day varied from 3.0 Gm. for the 18 month old infant to 10.0 Gm. for the adults. The

TABLE 1.—*Patients with Meningococcic Meningitis Treated with Gantrisin® Alone*

						"Gantrisin"						
Patient	Age, Year	Day of Illness Admitted	Spinal Fluid Cell Count, per Cu. Mm.	Blood Culture	Urinary Findings	Initial Intravenous Dose, Gm.	Daily Dose, Gm.	Days Taken	Blood Free, Mg./100 Cc.	Levels Total, Mg./100 Cc.	Duration of Fever, Days	Outcome
J. B.	24	3	30,000	+	Albumin, 4+; later cleared	6	10 I. V.; maintenance, 6 oral	10	...	...	6	Recovered
E. D.	18	2	20,000	..	Red blood cells at onset; cleared	6	8 I. V.; maintenance, 6 I. V., 6 oral	10	5.2 3.2	6.4 3.7	7	Recovered
D. R.	6	3	1,400	—	Albumin, 4+; later cleared	4	6 oral	6	...	...	2	Recovered
L. S.	35	2	15,000	—	Albumin, 4+; later cleared	3	6 oral	12	...	...	5	Recovered
G. R.	4	2	.....	+	Albumin, 1+; later cleared	3	4.5 oral	6	...	...	3	Recovered
R. R.	19	?	950	—	Crystals on 8th day; cleared spontaneously	4	6 oral	12	...	...	9	Recovered
L. D.	6	2	12,800	—	Albumin at onset; cleared	3	3 oral	11	9.9 12.8	11.5 14.9	9	Recovered
S. S.	1½	3	4,770	..	Normal at onset; crystals on 6th day; cleared spontaneously	3	4 I. V., 4 oral	12	7.4	10.5	3	Recovered
R. S.	5	2	6,700	..	Albumin at onset, red blood cells throughout treatment	3	6 I. V., 6 oral	8	6.7	10.2	4	Recovered
L. G.*	42	3	750	—	Albumin (1+) and red blood cells on admission; albumin (1+) on discharge	4	6 I. V.	9	6.7 14.5 18.0	8.2 19.4 22.6	4	Recovered

* Meningococci not found in spinal fluid or blood culture. Typical petechial hemorrhages of meningococcemia.

TABLE 2.—*Patients with Meningococcic Meningitis Treated with Gantrisin® and Penicillin*

						"Gantrisin"								
Patient	Age, Year	Day of Illness Admitted	Spinal Fluid Cell Count, per Cu. Mm.	Blood Culture	Urinary Findings	Initial Intravenous Dose, Gm.	Daily Dose, Gm.	Days Taken	Blood Free, Mg./100 Cc.	Levels Total, Mg./100 Cc.	Duration of Fever, Days	Penicillin Units Daily	Complications	Outcome
K. I.	6	2	40,000	—	Red blood cells (4+) at onset; later cleared	3	4 oral	5	9.2	12.0	5	240,000	None	Recovered
L. F.	3	1	6,300	+	Albumin at onset; cleared	4	3 oral	7	...	....	4	160,000	None	Recovered

total daily maintenance dose averaged 6.0 Gm., given at four hour intervals in doses of 1.0 Gm. each, for adults and 3.0 or 4.0 Gm. for children. Often, when the intravenous route was used, 2.0 or 3.0 Gm. were given every eight hours instead of smaller doses every four hours. The

administration of gantrisin® was continued three to nine days after the temperature reached the normal mark.

Two patients with meningococcic meningitis were treated with gantrisin® in combination with penicillin. Both recovered.

CASE 1.—For K. I. (table 2), the administration of penicillin in doses of 30,000 units in aqueous solution at three hour intervals was begun on admission. On the third hospital day his condition was considered critical. The administration of gantrisin® in 2.0 Gm. doses intravenously at eight hour intervals was then begun. Later the penicillin was mixed with 5 cc. of 20 per cent solution of the sulfonamide salt, and the agents were injected intramuscularly together at four hour intervals.

CASE 2.—The second patient (L. F., table 2) received penicillin and gantrisin® in combination from the time of admission. Both patients made an uneventful recovery.

TABLE 3.—*Patients with Pneumococcic Meningitis Treated with Gantrisin® and Penicillin*

						"Gantrisin"							
Patient	Age, Year	Day of Illness Admitted	Spinal Fluid Cell Count, per Cu. Mm.	Blood Culture	Urinary Findings	Initial Intravenous Dose, Gm.	Daily Dose, Gm.	Days Taken	Blood Free, Mg./100 Cc.	Levels Total, Mg./100 Cc.	Duration of Fever, Days	Penicillin, Units Daily	Complications	Outcome
P. McG.	41	4	9,000	+	Albumin (4+) at onset; later cleared	4	6 I. V., 6 oral	11	12.4	17.9	6	320,000	None	Recovered
H. M.	50	?	250	+	Albumin (3+) throughout course	6	6 oral	16	....	....	16	800,000	Bacterial endocarditis	Died
M. O'C.	51	2	3,700	—	Normal	4	6 oral	24	11.5	17.0	14	400,000	Mastoiditis	Recovered

PNEUMOCOCCIC MENINGITIS

The patients with pneumococcic meningitis were so ill on admission and the outlook for patients with this disease is known to be so bad that we felt compelled to give them the combined treatment.

CASE 3.—F. McG. (table 3) had entered Cook County Hospital two months prior to the attack of meningitis because of dyspnea and precordial pain. The blood pressure was 240 systolic and 140 diastolic, the specific gravity of the urine was fixed between 1.005 and 1.009 and the urea clearance was 22 per cent of normal. The final diagnosis at that time was chronic glomerulonephritis. The patient was readmitted because of vomiting, headache, backache and fever and soon went into coma. The urine contained albumin (3 plus) and many erythrocytes on admission. The average dose of penicillin was 40,000 units in aqueous solution, given intramuscularly every three hours, and for five days an intrathecal dose of 10,000 units was given every twenty-four hours. Starting three days after admission gantrisin® was used in doses of 6.0 Gm. daily, given intravenously for five days and then orally for five days longer. Because of the known renal insufficiency and microscopic hematuria the urine was kept alkaline from the start. No red blood cells were found in the urine after the first hospital day, and the patient recovered.

CASE 4.—H. M. (table 3) was admitted first to a ward of Cook County Hospital with the diagnosis of pneumonia of the lower lobe of the left lung. She was found to have petechiae scattered over her body, and a presystolic apical murmur and

auricular fibrillation were present. Because of the cloudy mental state and the stiffness of the neck the patient was transferred to the contagious disease unit of Cook County Hospital. Although the spinal fluid contained only 250 cells, cultures from it and from the blood revealed pneumococci. In a previous report [2] we pointed out that a low spinal fluid cell count in pneumococcic meningitis is a serious prognostic sign. Also, in our previous experience, when the meningitis was associated with active pneumococcic endocarditis the patients had all died, regardless of the kind of treatment used. Treatment in this case consisted of the intravenous administration of 2.5 Gm. of gantrisin® every eight hours until the patient died, on the fourteenth hospital day. Beginning on the fourth day after admission she received, in addition, 800,000 units of penicillin in aqueous solution, given in divided doses intramuscularly daily. The autopsy revealed extensive pneumonia of the entire right lung, active endocarditis superimposed on mitral stenosis due to rheumatic heart disease, acute glomerulitis and chronic arterial and arteriolar nephrosclerosis, hemorrhages into the adrenal cortex and active meningitis.

TABLE 4.—*Patients with H. Influenzae Meningitis Treated with Gantrisin® and Streptomycin*

Patient	Age	Day of Illness Admitted	Spinal Fluid Cell Count, per Cu. Mm.	Blood Culture	Urinary Findings	"Gantrisin" Initial Dose, Gm.	"Gantrisin" Daily Dose, Gm.	"Gantrisin" Days Taken	Duration of Fever, Days	Streptomycin, Mg. Daily	Outcome
A. W.	4 yr.	2	2,000	—	Normal	2.0 I. V.; 7.0 total 1st day	6 I. V., 3 oral	10	12	800	Recovered
A. B.	20 mo.	9	30,000	..	Normal at onset; red blood cells twice later (cleared spontaneously)	2.0 oral	3 oral	22	4	500	Recovered

CASE 5.—M. O'C. (table 3) had pneumococcic meningitis secondary to mastoiditis, but without respiratory, cardiac or renal involvement. She made an uneventful recovery on combined treatment.

HEMOPHILUS INFLUENZAE MENINGITIS

In the 2 cases of meningitis due to H. influenzae in this series, treatment with gantrisin® was combined with injections of streptomycin.

CASE 6.—A. W. (table 4) was given the sulfonamide intravenously in 2.0 Gm. doses at eight hour intervals at the start. Later 0.5 Gm. doses every four hours were administered orally as the maintenance dose. On the eighth day after admission the patient was rational and nuchal rigidity was much less, but the temperature was still elevated to 102 F.; hence, the administration of streptomycin, 0.100 Gm. by deep hypodermic injection every three hours, was begun. After three more days on combined treatment the fever began to recede and the patient went on to uneventful recovery.

CASE 7.—The other patient with H. influenzae meningitis (A. B., table 4) was a 20 month old infant brought to Cook County Contagious Hospital on the ninth

2. Rhoads, P. S.; Hoyne, A. L.; Levin, B.; Horswell, R. G.; Reals, W. H., and Fox, W. W.: Treatment of Pneumococcic Meningitis, J. A. M. A. **115**:917-922 (Sept. 14) 1940.

day of the illness, which started with a cold accompanied with lethargy, nausea and fever. Nuchal rigidity and semicoma had been present two days when the patient was admitted. The temperature was 104 F. rectally on admission. H. influenzae, type B, was found in the spinal fluid. Four grams of gantrisin® were given orally the first day, and a maintenance dose of 3.0 Gm. daily by the same route was continued. On the third day the patient was improved, but the temperature was 101 F. Administration of streptomycin, 500 mg. daily divided into three hourly doses, was begun, and both drugs were continued for eighteen days more, although the patient was free of fever and all symptoms by the twelfth hospital day. Microscopic hematuria was found on the fourth and fourteenth hospital days, but the administration of gantrisin® was continued without change, and the red blood cells disappeared from the urine.

COMMENT

The patients received 2 to 6 Gm. of gantrisin® as the initial dose, but the average maintenance dose for adults was 6.0 Gm. daily, administered at four hour intervals. The blood levels of the drug averaged 9.45 mg. per hundred cubic centimeters free and 12.5 mg. total. Thus, 23.2 per cent of the gantrisin® was present in the conjugated form. The spinal fluid levels averaged one third to one half of the blood levels in specimens taken at the same time.

In 10 of the 17 patients of the entire series albuminuria was present before treatment; 5 of these had microscopic hematuria. In 8 the albumin had disappeared from the urine at the time of discharge. One patient with red blood cells in the urine at the start still had them at the time of discharge. The other conditions cleared up as treatment progressed. Crystals were noted in the urine of 1 patient on the sixth day and in that of another on the eighth day. Treatment was continued, and in both patients the crystalluria disappeared. One patient (F. McG, table 3) known to have chronic glomerular nephritis received sodium bicarbonate in doses up to 18 Gm. daily for the first six days of treatment with gantrisin.® None of the others received any alkali as adjuvant treatment.

Since in only 1 patient was the microscopic hematuria discovered after the sulfonamide treatment was begun, it is unlikely that it was responsible for any renal bleeding except in this 1 instance. In this instance the relationship is conjectural only, since the hematuria cleared up while further doses of gantrisin® were being administered.

No other reactions suspected of being due to the sulfonamide were encountered.

SUMMARY

Ten patients with meningococcic meningitis were treated with a new sulfonamide, gantrisin,® which was used as the sole bacteriostatic agent. All recovered without complications due to the disease or the treatment.

Two patients with meningococcic meningitis were given combined treatment with gantrisin® and penicillin. Both recovered without complications.

Three patients with pneumococcic meningitis were treated with gantrisin® and penicillin. One of these, shown by autopsy to have pneumococcic endocarditis, died. The others recovered.

Two patients with H. influenzae meningitis were treated with gantrisin® and streptomycin. Both made an uneventful recovery.

Gantrisin® was administered orally, intramuscularly and intravenously with no untoward effects. Several patients who had albuminuria and microscopic hematuria at the start of treatment with gantrisin® were given the drug in full doses without adjuvant alkali therapy, and these conditions cleared up under treatment.

MULTIPLE SYMMETRIC BILATERAL CRANIAL NERVE PALSIES IN PATIENTS WITH UNREGULATED DIABETES MELLITUS

Report of Three Cases

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PERIPHERAL neuropathy in patients with diabetes mellitus is not uncommon, and several reviews of the subject have appeared in recent years.[1] The mechanism of production of the neuritis has not been definitely established, but it appears to bear some relation to inadequate regulation, since it is reputed to be less frequent in patients in whom the diabetes has been continuously and carefully controlled. The onset is usually characterized by the gradual development of paresthesias and motor weakness; however, these symptoms may have a precipitous onset in the presence of acidosis or of a complicating acute infection.[1b] The lower extremities are commonly more severely affected than are the upper ones, and the neurologic findings in general are those of a mixed polyneuropathy involving the motor and sensory nerves.

Paralysis of the cranial nerves in diabetes was first described by Ogle[2] in 1866. Since then, numerous case reports have appeared, and Jordan[1a] has reviewed the older literature on this subject. The coexistence of neuropathy of cranial and peripheral nerves due to diabetes has been infrequently mentioned.[3] The nerves of the extraocular muscles are most frequently affected,[4] but, as Jordan pointed out, optic neuritis,

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1. (*a*) Jordan, W. R.: Neuritic Manifestations in Diabetes Mellitus, Arch. Int. Med. **57**:307 (Feb.) 1936. (*b*) Rundles, R. W.: Diabetic Neuropathy, Medicine **24**:111, 1945. (*c*) Broch, O. J., and Klövstad, O.: Polyneuritis in Diabetes Mellitus, Acta med. Scandinav. **127**:514, 1947.

2. Cited by Collier, J.: Paralysis of Oculomotor Nerve-Trunks in Diabetes, Proc. Roy. Soc. Med. (Sect. Neurol.) **23**:627, 1930.

3. Root, H. F.: Paralysis of the External Ocular Muscles in Diabetes, M. Clin. North America **16**:985, 1933.

4. (*a*) Cited by Collier.[2] (*b*) Root.[3] (*c*) Dieulafoy: Paralysie des nerfs moteurs de l'œil chez les diabétiques, Presse méd. **13**:713, 1905. (*d*) Weinstein, E. A., and Dolger, H.: Extraocular Muscle Palsies Occurring in Diabetes Mellitus, Tr. Am. Neurol. A. **72**:199, 1947.

pupillary changes, facial paralysis and deafness may occur. Isolated instances of trigeminal involvement [5] have also been reported, although the diabetic causation is open to question. Weinstein and Dolger [4d] analyzed the records of 11 patients with diabetes with palsies of extraocular muscles. They concluded that the neurologic findings are best explained on the basis of small hemorrhages in the nuclei of the third nerve. The paralyses of cranial nerves are usually unilateral or asymmetric; bilateral, symmetric involvement has been mentioned only infrequently.[6] In 2 of Ogle's patients [2] bilateral paralysis of the third nerve developed shortly before death in acidosis. In a personal communication, Dr. Howard F. Root stated that he had recently observed facial diplegia in association with an episode of diabetic acidosis.

It is the purpose of the present communication to report extensive bilateral, symmetric palsies of cranial nerves in 3 patients with inadequately controlled diabetes mellitus. No records have been found in the literature of observation of comparable involvement of cranial nerves in this disease.

REPORT OF CASES

CASE 1.—R. D., a 36 year old Negro housewife, was admitted to the Presbyterian Hospital in severe acidosis. She had been known to have diabetes for ten years and for the eight years preceding admission had been in good general health. During this time she had been under no medical supervision and had maintained herself on 30 units of insulin daily. One week prior to admission she experienced a mild infection of the upper respiratory tract. The patient was otherwise well until three days before admission, when headache and backache developed. On the following day she lost her appetite and discontinued the use of insulin. On the day of admission she began to vomit.

Physical Examination.—On admission the patient was dehydrated, restless and disoriented. The temperature was 98.4 F., the pulse rate 96, the respiration rate 28 and the blood pressure 130 systolic and 60 diastolic. She had bilateral subconjunctival hemorrhages and early bilateral cataracts but no diabetic retinopathy. There was mild pharyngeal injection. A few fine, moist rales were heard over the lower lobe of the right lung. The heart and abdomen were normal. A complete physical examination three days after admission revealed no significant change; the reflexes were slightly hypoactive.

Laboratory Data.—The hemoglobin concentration was 12 Gm., and the red blood cell count, 3,800,000; the white blood cell count was 9,500, with 72 per cent polymorphonuclear leukocytes, 25 per cent lymphocytes, 2 per cent monocytes and 1 per cent basophils. The urinalysis revealed the following values: specific gravity, 1.015; albumin, 2 plus; glucose, 4 plus; acetone, 4 plus, and diacetic acid, 2 plus; there were occasional white blood cells and 2 to 3 granular casts per high power field. The erythrocyte sedimentation rate was 40 mm. in one hour, the carbon

5. Salomonson, W.: Sur l'absence de reflexes patellaires dans le diabète sucré, Centrabl. f. Nervenh. u. Psychiat. 1891, p. 160.

6. Cited by Collier.[2] Villard, H.; Viallefont, H., and Bouzigues: Double paralysie du moteur oculaire externe chez un diabétique, Rev. d'oto-neuro-opht. **12**:425, 1934.

dioxide capacity of the blood 16 volumes per cent and the blood sugar level 400 mg. per hundred cubic centimeters. The Kline reaction was negative. The Wassermann and colloidal gold reactions of the cerebrospinal fluid were negative, but the protein content was increased, as indicated in the table. Hemolytic streptococci were recovered from the throat culture. The electrocardiogram was interpreted as indicating only sinus tachycardia; the roentgenogram of the chest was noncontributory.

Course.—Despite prompt initial response to the usual measures directed against diabetic acidosis, the patient frequently exhibited transient ketonuria and glycosuria throughout the four months of hospitalization. On the fourth hospital day the patient experienced circumoral anesthesia and on the sixth day numbness over the entire body. During the second hospital week thickness of speech and drooling developed; this was accompanied by impairment of facial movements, mastication

Summary of Data in 3 Cases of Palsies of Cranial Nerves and Diabetes Mellitus

Patient	Diabetes	Precipitating Factor	Onset of Neuropathy	Spinal Fluid Protein, Mg./100 Cc.	Spinal Fluid Lymphocytes, Cells/Cu. Mm.	Cranial Nerves Involved
R. D., 33 yr., ♀	10 years' duration; inadequate regulation	Diabetic acidosis after respiratory infection	3 days after onset of acidosis; 11 days after onset of respiratory infection	At onset, 88 Maximum, 150 At discharge, 33	At onset, 7 Maximum, 12 At discharge, 3	III, V, VII, IX, X (bilateral involvement)
F. B., 33 yr., ♀	4 years' duration; diabetic acidosis 4 months before admission; inadequate regulation	Withdrawal of insulin for 3 days before admission diabetic coma	8 days after onset of acidosis	At onset, 45 Maximum, 104	At onset, 0 Maximum, 11	III, V, VII, IX, X (bilateral involvement); VI (left)
M. A., 61 yr., ♀	12 years' duration; inadequate regulation	Diabetic ketosis after respiratory infection	At onset of respiratory infection	At onset, 48 Maximum, 86 At discharge, 58	At onset, 0 Maximum, 28 At discharge, 4	III, V, VI, VII, IX, X, XI (bilateral involvement)

and deglutition. On neurologic examination during this period the patient was noted to have fixed miotic pupils and bilateral weakness of muscles innervated by the fifth, seventh, ninth and tenth cranial nerves, with hypesthesia over the distribution of the sensory division of the fifth, ninth and tenth nerves. She also demonstrated generalized hypesthesia to pinprick, generalized muscular weakness and diminished deep tendon reflexes. After the onset of the palsies of cranial nerves the patient began to complain of pains in the lower extremities. Neurologic signs and symptoms fluctuated somewhat until the fourth hospital week, when the patient began to improve progressively. Seven months after onset of illness neurologic examination was noncontributory except that it revealed residual hyporeflexia, slight bilateral weakness of the facial nerve and deviation of the uvula to the left. Cerebrospinal protein values and lymphocyte counts were elevated in the active phase of the neuropathy but returned to normal as symptoms and signs subsided, as indicated in the table. Since discharge from the hospital the patient has attended the clinic. At the time of writing she is in good general health. The diabetes is satisfactorily controlled on 37 units of insulin daily, and she no longer has abnormal neurologic symptoms or signs.

CASE 2.—F. B., a Negro woman of 33 years, was first admitted to the Presbyterian Hospital in July 1947 for the removal of a nontoxic nodular goiter. She had been known to have diabetes for four years at that time; she had been maintained without symptoms on 35 to 40 units of insulin daily but without dietary restrictions or examinations of the urine. After an uneventful partial thyroidectomy she remained under clinic supervision for eight weeks; the diabetes was inadequately controlled on 32 units of insulin daily. At the end of that time she was readmitted to the hospital in severe diabetic acidosis. She responded rapidly to the usual measures, and there were no neurologic complications. After discharge unsatisfactory control of the diabetes persisted as before, with both glycosuria and insulin shocks. Three months later she was readmitted with a history of three days of withdrawal of insulin and eighteen hours of semicoma. There was no history of infection or any other recognized precipitating event.

Physical Examination.—On admission the patient was dehydrated and semicomatose, responding only to painful stimuli; the respirations were of Kussmaul type. The temperature was 96.6 F., the pulse rate 120 and the respiration rate 32; the systolic blood pressure was 75, and the diastolic blood pressure could not be determined. The pupils were fixed, but no ocular palsy or retinopathy was present. The lungs, heart, abdomen and extremities were normal; the deep tendon reflexes were absent except for the knee jerks, which were diminished.

Laboratory Data.—The hemoglobin content was 14.5 Gm. and the red blood cell count 4,900,000; the white blood cell count was 26,000, with 89 per cent polymorphonuclear leukocytes and 11 per cent lymphocytes. The urinalysis showed the following values: specific gravity, 1.022; glucose, 4 plus; acetone, 4 plus, and diacetic acid, 4 plus; there were 4 to 6 white blood cells per high power field. The hematocrit reading was 50 per cent, the carbon dioxide-combing power of the blood 11 volumes per cent and the blood sugar level 733 mg., and the blood urea nitrogen level 77 mg., per hundred cubic centimeters. The electrocardiogram was interpreted as showing definite evidence of myocardial damage; the roentgenogram of the chest was noncontributory. On examination when the patient was admitted the cerebrospinal fluid was normal in all respects, including Wassermann and colloidal gold reactions.

Course.—The diabetic ketosis and acidosis cleared within twenty-four hours, but full recovery of consciousness did not occur for fifty-six hours. By the third hospital day general physical examination gave normal results. As in case 1, diabetes was difficult to control throughout the three months' hospitalization, as evidenced by occasional insulin shocks, intermittent glycosuria and occasional acetonuria. There were no further abnormalities noted until the eighth hospital day, when numbness of the face (particularly about the lips), difficulty in chewing, difficulty in swallowing both liquids and solid food and drooling of saliva developed. On examination during the second hospital week the patient showed bilaterally fixed pupils, impaired convergence, weakness of the sixth nerve on the left side and bilateral weakness of the seventh nerve, hypesthesia over the distribution of the fifth nerve bilaterally, generalized motor weakness, absence of deep tendon reflexes in the lower extremities and normal sensory conditions aside from the face. Cerebrospinal fluid withdrawn at this time showed an increase in protein to 104 mg. per hundred cubic centimeters, with 11 lymphocytes present, as indicated in the table. The course was one of gradual improvement, but, as in case 1, pains in the extremities were a prominent and persistent complaint. The neuropathy did not clear entirely while the patient was in the hospital, but six months after the onset of the illness she was free from subjective complaints. A complete

neurologic examination one year after discharge gave normal results except for sluggish pupillary reactions and poor convergence. The patient was seen frequently in the clinic after discharge from the hospital, and the diabetes appeared to be well controlled on 27 units of insulin daily.

CASE 3.—M. A., an Italian housewife of 61 years, was known to have had diabetes for at least twelve years. She had been treated with dietary measures alone for the first four years, but over the next eight years she had taken 25 to 30 units of insulin daily without any dietary restrictions. She had never been closely supervised but had had no insulin shocks or acidosis. Ten days before admission, while otherwise in good general health, she experienced the onset of symptoms suggesting a respiratory infection, accompanied with mild general weakness. On the same day the patient first noted difficulty in swallowing; this symptom progressed until, at the time of admission, she could swallow only liquids in small amounts. Eight days before admission bilateral ptosis appeared, and dysarthria, headache, feverish sensations and urinary hesitancy developed; the patient continued to take insulin throughout this period.

Physical Examination.—On admission the patient appeared dehydrated and acutely ill, with a temperature of 101 F., a pulse rate of 74, a respiration rate of 20 and a blood pressure of 130 systolic and 80 diastolic. Early cataracts were present, with minimal arteriosclerotic changes in the fundi. There was an area of crusted exudate over the hard palate and the posterior pharyngeal wall. The patient demonstrated little expansion of the thorax, and an expiratory stridor was present. The lungs were clear, and the examination of the heart and abdomen was noncontributory, as was the pelvic examination. The patient exhibited ptosis bilaterally with 60 per cent narrowing of the palpebral fissures, masklike facies and thick speech, and she complained of difficulty in swallowing. Further neurologic examination revealed severe generalized muscular weakness and diminished deep tendon reflexes in the lower extremities. The remainder of the examination, including that of the sensory modalities, was noncontributory.

Laboratory Data.—The hemoglobin content was 13.5 Gm. and the red blood cell count 4,200,000; the white blood cell count was 6,800, with 61 per cent polymorphonuclear leukocytes, 35 per cent lymphocytes, 2 per cent eosinophils, 1 per cent monocytes and 1 per cent basophils. The specific gravity of the urine was 1.023, and the following values were noted: glucose, 2 plus; acetone, 3 plus, and diacetic acid, 2 plus; there were 1 to 3 white blood cells and 3 to 5 red blood cells per high power field. The erythrocyte sedimentation rate was 50 mm. in one hour; the hematocrit reading, 36 per cent; the carbon dioxide content of the blood, 70 volumes per cent; the blood sugar level, 116 mg. per hundred cubic centimeters; the serum chloride content, 103.2 milliequivalents; the sodium content, 141.5 milliequivalents; the potassium, 3.6 milliequivalents, and the blood urea nitrogen level, 15 mg. per hundred cubic centimeters. The Kline reaction was negative. A few colonies of hemolytic streptococci were recovered from the throat culture, and no Mycobacterium diphtheriae could be demonstrated. The electrocardiogram was interpreted as within normal limits, and the roentgenogram of the chest showed no evidence of recent infiltration. The cerebrospinal fluid on admission was normal in all respects, including the Wassermann and colloidal gold reactions.

In contrast to the 2 preceding cases, control of the diabetes was satisfactory throughout the patient's hospital course. On a low caloric intake the insulin requirement was 50 units daily. There was no improvement in the muscular weakness after the subcutaneous administration of neostigmine methylsulfate (prostig-

mine methylsulfate®) in doses up to 2.5 mg. Chronaxia studies of the upper and lower extremities showed partial denervation of the small muscles of the hands, the extensors of the fingers and the deltoid and brachioradialis muscles, together with many of the muscles of the lower extremities. Muscles supplied by the fifth, seventh and eleventh cranial nerves showed similar changes. The involvement of the fifth and eleventh cranial nerves had not been suspected clinically. The chronaxia studies were interpreted as consistent with polyneuritis. Electromyographic studies of the muscles of the upper arm, the forearm and the intrinsic muscles of the hand were interpreted as indicating widespread involvement of the lower motor neurons, with a moderate amount of atrophy in progress. These measurements were repeated four weeks later and showed no significant change.

The patient was fed by gavage during the first two hospital weeks with gradual improvement in all signs and symptoms, so that at the end of that time she was able to swallow soft food. Cerebrospinal protein values were not found to be elevated until the second hospital week, as shown in the table, but they then remained elevated up to the time of discharge from the hospital. At the end of six weeks of hospitalization the patient was well except for mild generalized muscular weakness, and aside from this the neurologic examination gave essentially normal results.

COMMENT

The similarity of the 3 cases described seems to be sufficiently great to warrant the assumption that the neuropathic signs were probably of similar causation. All 3 patients had diabetes of four to twelve years' duration, requiring moderate amounts of insulin. In each case there was a background either of poor diabetic control or of inadequate supervision, and 2 of the patients exhibited markedly labile diabetes throughout the hospital course. In no case was there a history of antecedent neurologic disease, and in 2 cases the neurologic examinations performed on admission were noncontributory. In all 3 patients bilateral symmetric multiple palsies of cranial nerves developed rapidly; in 2 patients, they made their first appearance after the correction of severe diabetic acidosis. In each case severe polyneuropathy involving the peripheral nerves began at the same time or followed the onset of the neuropathy of the cranial nerves. At some time during the acute illness, spinal fluid protein values were moderately elevated while the number of lymphocytes was only slightly raised. In all cases, recovery was essentially complete within three months. There were residual pains in the extremities in cases 1 and 2, but these subsequently disappeared over the course of several months. After discharge from the hospital the patients attended the clinic, and satisfactory diabetic regulation has been achieved at the time of this report.

Multiple bilateral involvement of cranial nerves may be found in association with the polyneuropathy of peripheral nerves which follows diphtheria[7] and in the "infectious" type of polyneuropathy usually

7. Wilson, S. A. K.: Neurology, edited by A. N. Bruce, ed. 1, Baltimore, Williams & Wilkins Company, vol. 1, p. 639, 1940.

referred to as the Guillain-Barré-Stohl syndrome.[8] Paralyses of cranial nerves are rare in polyneuropathies owing to exposure to heavy metals, ingestion of alcohol or nutritional deficiency; these types are usually identified by the history and by the presence of characteristic neurologic findings. Involvement of the cranial nerves in diphtheritic polyneuritis is usually ushered in with palatal weakness and difficulty in convergence. In patient 3 palatal weakness and failure of convergence were severe and were the earliest neurologic symptoms, but cultures of the nose and throat for Myco. diphtheriae were negative. In patient 1 there was no involvement of extraocular muscles and only palatal deviation was present; patient 2 had impaired convergence and weakness of the sixth cranial nerve on the left side. In these 2 cases no examination for Myco. diphtheriae was performed, but neither neurologic nor clinical features were thought to be suggestive of diphtheria.

Whereas bilateral weakness of cranial nerves may occasionally occur after diphtheria, it is a common finding in patients with so-called infectious polyneuritis. The latter, according to Brown and Baker,[9] is considered to be an exceedingly variable syndrome. Each of the cases reported here conceivably could fall into this group and would undoubtedly so be classified were it not for the presence of diabetes mellitus. Unfortunately, it is impossible to establish conclusively a diabetic causation for the polyneuritis in any given case. However, it seems unlikely that these 3 cases represent coexistent infectious polyneuritis and diabetes.

SUMMARY

Three cases are reported in which extensive bilateral symmetric palsies of cranial nerves, together with polyneuropathy of peripheral nerves, developed rapidly during the course of unregulated diabetes mellitus. Complete recovery from the neurologic disturbances was observed after satisfactory regulation.

8. Forster, F. M.; Brown, M., and Merritt, H. H.: Polyneuritis with Facial Diplegia, New England J. Med. **225**:51, 1941.

9. Brown, J. R., and Baker, A. B.: The Diagnosis of Guillain-Barré's Disease, Am. J. Med. **2**:45, 1947.

RELATION BETWEEN BLOOD LIPIDS AND EXCRETION OF CHOLINE IN DIABETIC PATIENTS

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THE INCREASE of blood lipid fractions, diabetic–hyperlipemia, is a well known sign of the abnormal fat metabolism which takes place in a system affected with diabetes mellitus.

The blood lipids can be divided into three groups, composed of (1) neutral fat, (2) phospholipids and (3) cholesterol. Recent studies seem to indicate that practically all phospholipids contain choline,[1] that is to say, they are composed of sphingomyelin and, mainly, of lecithin. Choline is regarded as an important dietary factor, playing an essential role in fat metabolism.[2] In this laboratory the excretion of choline in the urine of persons with diabetes was investigated, and an increased choline output was found in some instances.[3] In continuing these investigations, the relationship between the amount of choline excreted in the urine and the diabetic lipemia has been studied.

MATERIAL

Twelve patients with diabetes, 5 men and 7 women, ranging in age from 24 to 57 years with an average of 39, served as subjects for this investigation.[4] A history was taken and a complete clinical examination made in order to eliminate subjects with other conditions which are also known to affect the level of blood lipids (infections, cancer, thyroid dysfunction, etc.). The patients were given a hospital diet corresponding to common Swedish food, without restrictions regarding the amount and the composition. The experiments were usually carried out during the last days of hospitalization, when the patients had

From the Department of Metabolic Research, Wenner-Grens Institute, Stockholm University.

1. Taurog, A.; Entenman, C., and Chaikoff, I. L.: The Choline-Containing and Non-Choline Containing Phospholipids of Plasma, J. Biol. Chem. **156**:385, 1944. Entenman, C., and Chaikoff, I. L.: On the Determination of Choline in Liver and Plasma of the Dog, ibid. **160**:377, 1945.

2. Širek, O. V.: Choline as a Lipotropic Factor, Bratisl. lek. listy **29**:187, 1949.

3. Csáky, T. Z.; Möllerström, J., and Širek, O. V.: The Excretion of Choline in the Urine of Diabetic Patients, Arch. Int. Med. **84**:730 (Nov.) 1949.

4. The patients were from the Research Clinic for Diabetes, annexed to the Department of Metabolic Research.

attained, as far as possible, a metabolic equilibrium with administration of sufficient amounts of insulin. Over a period of three days the twenty-four hour urine output was collected and the choline content estimated. The third morning about 9 a. m., after a fasting period of ten to twelve hours, 20 cc. of blood was collected and the blood lipids in the plasma estimated (as an anticoagulant 2 drops of a 1 per cent solution of heparin sodium were used). The subjects had eaten nothing since the previous evening meal, had indulged in no exercise and were not emotionally disturbed by the procedure. The women patients were always tested about two weeks after a menstrual period. In the literature dealing with concentrations of blood lipid fractions, there is much uncertainty as to the normal range of blood fats in the post-absorptive state.[5] For this reason control determinations of blood lipids in normal subjects after the same fasting period, and on the same diet, were carried out. Estimations of choline in these normal subjects were not carried out, as it has been shown that the daily urinary excretion in normal persons shows variations up to 5 mg.[6]

METHODS

Determination of Choline in the Urine.—This was carried out in the same way as described previously.[8] The principle of this method is the acetylation of the choline into the more active acetylcholine and the determination of the latter by pharmacologic titration on surviving rabbit intestine. The standard error of a single determination was ± 10 per cent.

Determination of Total Fat (or Total Lipids).—This was carried out by means of the gravimetric method, according to the description given by Brun.[7] The principle is the precipitation of proteins in a measured volume of plasma (2 cc.) with an alcohol-acetone solution, according to the method of Schoenheimer and Sperry.[8] The protein-free filtrate is evaporated, dissolved in petroleum ether, filtered and evaporated in a glass dish of known weight. The difference between the weight of the clean glass dish and its weight after the evaporation of the petroleum ether extract indicates the amount of total fat in milligrams. Each determination was made twice, and the standard error of the mean of a double estimation was ± 4 per cent.

A number of microlipid methods were considered for the determination of all the lipids as yet known to be present in the blood plasma. The chemical oxidation

5. (*a*) Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry Interpretation, Baltimore, Williams & Wilkins Company, 1946, vol. 1, pp. 373-544. (*b*) Man, E. B., and Gildea, E. F.: A Modification of the Stoddard and Drury Titrimetric Method for the Determination of the Fatty Acids in Blood Serum, J. Biol. Chem. **99**:43, 1932. (*c*) Boyd, E. M.: A Differential Lipid Analysis of Blood Plasma in Normal Young Women by Microexidative Methods, ibid. **101**: 323, 1933.

6. (*a*) Csáky, Möllerström and Širek.[8] (*b*) Borglin, N. E.: On the Excretion of Choline in the Urine, Acta pharmacol. et toxicol. (supp. 1) **3**:95, 1947.

7. Brun, G.: Determination of Total Fat in Serum, Bibliot. f. læger **131**:197, 1939.

8. Schoenheimer, R., and Sperry, W. M.: A Micromethod for the Determination of Free and Combined Cholesterol, J. Biol. Chem. **106**:745, 1934.

method is generally regarded as the most reliable, giving the true values for total fat. The gravimetric method selected in our experiments has the advantage of being very simple as compared with the chemical method and has proved suitable for routine determinations in the clinical laboratory. The gravimetric analyses have the drawback that the fat can be contaminated with nonlipid materials and that, therefore, slightly higher values may be obtained. The reproducibility, however, is sufficient, and it will be shown that the figures obtained by this method in normal subjects did not differ appreciably from the physiologic ranges given by other authors.

Determination of Lipid Phosphorus.—This was carried out according to the description given by Brun.[9] The principle is the combustion of the aforementioned amount of total fat with concentrated sulfuric acid and concentrated nitric acid. The remainder is treated with molybdic acid, and the resulting phosphomolybdate is reduced with hydroquinone. The resulting blue color is checked in the Klett-Summerson photoelectric colorimeter. Double analyses were always carried out, and the standard error of the mean was ± 1.2 per cent.

In estimating the phospholipid content of the plasma, the conversion factor 25 was used (P × 25), as about 4 per cent of the lecithin molecule is composed of phosphorus, and most of the phospholipids derive from lecithin.[10]

Determination of Total Cholesterol.—This was carried out according to the method of Bloor, Pelkan and Allen,[11] with slight modifications. The principle of this method is the precipitation of a measured amount of plasma (0.5 cc.) with a 3:1 mixture of alcohol and ether. The liquid is filtered and evaporated. The dry material is then dissolved in chloroform and quantitatively transferred into the colorimeter tube. Finally, acetic anhydride and concentrated sulfuric acid are added and the yellow color read in the Klett-Summerson photoelectric colorimeter. Each determination was made twice, and the standard error of the mean of the double analysis was ± 1.4 per cent.

RESULTS

In table 1 are presented the results of choline estimations over a period of three consecutive days for 12 patients with diabetes. Seven patients showed an excretion of over 5 mg. per day, and the mean value for three days varied from 6.5 mg. to 10.34 mg. Five patients showed a normal excretion of choline, with mean values within physiologic limits up to 5.00 mg. a day.

It further emerges from table 1 that the choline excretion of one person shows large variations (e. g., A. K., for whom values of 5.55 mg., 14.47 mg. and 11.00 mg. per day were obtained), which are dependent mainly on the choline content of the food.[6b] It is therefore emphasized that it is impossible to obtain a true picture of the choline excretion when the rate for only one day is considered. Variations

9. Brun, G.: Determination of Lipoid Phosphorus in Serum, Bibliot. f. læger **131**:203, 1939.

10. Hejda, B., and others: Guide to Clinical Laboratory Methods, Prague, Jaroslav Tožička, 1940, pp. 109-110.

11. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma, J. Biol. Chem. **52**:191, 1922.

owing to the diet must be ruled out by computing the mean value of figures obtained by estimation of choline for several days. In my own experience, a reliable impression of the choline metabolism can only be secured by using the mean for at least three days.

The figures for total cholesterol, total lipids, lipid phosphorus and the amount of phospholipids expressed as lecithin (P × 25) in the blood plasma are given in table 2. Each figure is the mean of two parallel estimations. Three groups of values are presented. The first group includes figures for blood lipid fractions in persons with diabetes—who had increased choline excretion (later called group D); the second gives values for patients with choline excretion within physiologic limits (later called group d), and in the third group are presented figures of blood lipid fractions in normal subjects (later called group NP) for comparison. As to group NP, the total cholesterol content of the plasma varied in my material from 175 mg. to 270 mg. per hundred cubic centimeters of plasma, the average being 211 mg. The content of lipid phosphorus varied from 5.60 mg. to 8.23 mg. per hundred cubic centimeters of plasma, with an average of 7.47 mg. The phospholipid content, calculated as lecithin by means of the conversion factor 25, lay between 140.00 and 205.75 mg. per hundred cubic centimeters of plasma, the average being 151.75 mg. These results were checked with the most reliable figures from the literature.[5a, c] The figures for total fat analysis in my material were between 541 mg. and 850 mg. per hundred cubic centimeters of plasma, the average being 737 mg. The physiologic ranges reported by other authors, as far as they were established by dependable methods, lay between 320 mg. and 820 mg. per hundred cubic centimeters of plasma, an average of about 600 mg.[12] The figures obtained for total fat in my normal subjects thus tallied with those given in the literature, and only the average was higher, owing to the use of the gravimetric method, as detailed.

If one considers the other two groups, D and d, and compares the values for the various lipid fractions, it may be seen that the differences were marked for choline and lipid phosphorus (lecithin) but less for total fat and cholesterol. A good example is patient L. S., in group D, whose cholesterol level was 250 mg. (normal subject B. L. showed a cholesterol level of 270 mg.), whereas all the other values for L. S. were greatly increased. On the other hand, patient A. B., in group d, showed total fat of 906 mg. (the highest normal value in my material

12. (*a*) Peters and Van Slyke.[5a] (*b*) Boyd.[5c] (*c*) Vogt-Möller, P., and Lawaetz, B.: Studies on the Serviceability of the Hemolipocrit Method and on the Course of Alimentary Hyperlipemia in Normal Persons and in Patients Suffering from Idiopathic Steatorrhoea, Acta med. Scandinav. **92**:105, 1937. (*d*) Chamberlain, C. T.; Jacobs, S., and Butler, M. F.: Total Blood Fat Determinations as an Index of Thyroid Function, Am. J. Sc. **191**:66, 1936. (*e*) Man, E. B., and Gildea, E. F.: The Effect of the Ingestion of a Large Amount of Fat and of a Balanced Meal on the Blood Lipids of Normal Man, J. Biol. Chem. **99**:61, 1932.

TABLE 1.—*Excretion of Choline in the Urine of Diabetic Patients for Three Consecutive Days*

Patient....	N.J.	R.M.	W.H.H.	A.K.	L.S.	H.V.	S.A.C.	L.E.	O.M.S.	M.H.	B.H.	A.B.
Age, Yr....	32	24	28	51	47	51	43	57	31	48	24	24
Sex.....	♀	♀	♀	♂	♂	♂	♂	♂	♀	♀	♀	♀
Choline excretion, mg./day												
1st...	9.40	6.10	5.40	5.55	6.80	8.20	7.03	4.00	4.90	5.10	4.00	3.00
2d....	4.10	7.20	10.80	14.47	7.90	9.10	4.33	6.20	3.80	4.20	1.70	3.33
3d....	7.60	6.20	9.60	11.00	8.40	11.00	8.00	4.80	5.00	4.30	2.50	3.27
Averages..	7.03	6.50	8.60	10.34	7.70	9.43	6.75	5.00	4.56	4.53	2.73	3.20

TABLE 2.—*Plasma Lipids of Diabetic Patients and Normal Subjects in Postabsorptive State*

Patient	Sex	Type of Diabetes	Insulin Demand per Day, Units	Plasma Lipids, Mg./100 Cc. Cholesterol, Mg./100 Cc.	Total Fat	Lipid Phosphorus	Lecithin (P x 25)	Choline Mg./Day (Mean for 3 Days)
Patients with increased choline in urine								
N. J.	♀	Severe, ketogenic	88	290	1,287	13.63	340.75	7.03
R. M.	♀	Severe, ketogenic	96	280	1,288	13.00	325.00	6.50
W. A. M.	♀	Severe, ketogenic	72	390	1,001	11.00	275.00	8.60
A. K.	♂	Moderate, ketogenic	48	305	1,265	10.10	252.00	10.34
L. S.	♂	Moderate, ketogenic	72	250	1,230	11.43	285.00	7.70
H. V.	♂	Mild, nonketogenic	0	264	877	9.20	230.00	9.43
S. A. C.	♂	Moderate	64	253	840	9.14	228.50	6.75
Mean				319	1,125	11.07	276.75	8.05
Standard error*				27	77	0.65	16.25	0.57
Standard error, per cent				8.4	6.8	5.9	5.9	7.1
Patients with normal choline in urine								
L. E.	♂	Mild	0	300	925	8.79	214.75	5.00
O. M. S.	♀	Severe	80	250	810	8.51	212.75	4.56
M. H.	♀	Mild	12	260	843	8.40	210.00	4.53
B. M.	♀	Severe	48	296	820	8.05	201.25	2.73
A. B.	♀	Severe	80	230	905	7.60	190.00	3.20
Mean				267	860	8.27	206.75	4.00
Standard error				14	24	0.20	5.0	0.47
Standard error, per cent				5.1	2.9	2.4	2.4	11.75
Normal subjects								
G. M.	♀	....	...	190	736	5.60	140.00	....
P. S. E.	♂	....	...	220	800	6.50	162.50	....
V. S.	♂	....	...	175	655	7.00	117.25	....
R. U.	♀	....	....	206	850	7.94	198.50	....
B. L.	♀	....	...	270	850	8.10	202.50	....
L. F.	♀	....	...	215	796	8.11	202.75	....
S. O.	♂	....	...	195	541	8.20	205.00	....
L. B.	♀	....	...	220	669	8.23	205.75	....
Mean				211	737	7.47	151.75	....
Standard error				9	29	0.35	8.75	....
Standard error, per cent				4.2	5.2	4.7	4.7	....

* $\mu_{\bar{x}} = \dfrac{\left\{\dfrac{\Sigma\Delta x}{n}\right\} 1.25331}{\sqrt{n}}$

was 850 mg.), whereas the other figures for A. B. were definitely within physiologic limits. The level of lipid phosphorus (lecithin) in the blood and the figures for excreted choline showed a different picture. The highest value in group d (8.79 mg. for lipid phosphorus and 5.00 mg. for choline) was lower than the lowest figure in group D (9.14 mg. for lipid phosphorus and 6.75 for choline).

This observation was also statistically confirmed, as shown in table 3. The statistical evaluation of results for the three groups of subjects indicated significant differences between groups D and NP and groups D and d for lipid phosphorus and for choline, the values being 4.85, 4.12 and 5.51, but not for cholesterol and total lipids, as the rates were lower than 4. An exception was provided by the difference in total fat between groups D and NP, which was significant (4.52). The statistical differences in total fat and cholesterol between groups D and d

TABLE 3.—*Significant Differences in Cholesterol, Total Fat, Lipid Phosphorus and Choline Between Groups of Subjects* *

	$M \pm \mu r$ †	D‡	d§	NP¶
Cholesterol				
D.....	319± 27	....	1.71	3.78
d.....	267± 13.7	1.71	....	3.44
NP.....	211± 8.8	3.78	3.44	....
Total fat				
D.....	1125± 77	....	3.28	4.52
d.....	267± 13.7	3.28	....	2.69
NP.....	737± 38.6	4.52	2.69	....
Lipid phosphorus				
D.....	11.07± 0.65	....	4.12	4.85
d.....	8.27± 0.20	4.12	....	1.99
NP.....	7.47± 0.35	4.85	1.99	....
Choline				
D.....	8.05± 0.57	....	5.51	....
d.....	4.00± 0.47	5.51	....	....
NP.....	.....	....	....	....

* Significant difference $= \dfrac{M_1 - M_2}{\sqrt{\mu r_1^2 + \mu r_2^2}}$

† M is the mean; μr, the standard error of the mean.
‡ Diabetic patients with increased choline in urine.
§ Diabetic patients with normal choline in urine.
¶ Normal persons.

and groups D and NP were lower than 4, that is to say, in group D there were values for total fat and mainly cholesterol which fell in the normal physiologic range and, conversely, in group d there were those that rose over normal limits.

COMMENT

The methods adopted for determination of the choline in the urine [12] and of the lipid phosphorus and cholesterol in the blood plasma were well tested and the experimental errors negligible; the results obtained in normal subjects were in agreement with the findings of other authors.

Owing to technical difficulties, the level of free choline in the blood was not determined. The amounts of free choline are very small, viz.,

13. There is only free choline in the urine.[6b]

only about 0.5 mg. per hundred cubic centimeters of plasma,[14] and for double estimations 35 to 40 cc. of blood are required.

If one once more considers tables 2 and 3, it will be seen that increased urinary excretion of choline is usually followed by increased content of all the lipid constituents in the blood plasma. As significant differences between groups D and d were obtained only for choline (5.51) and for lipid phosphorus (4.12), one is obliged to assume that the relationship between choline and lipid phosphorus is closer than the relationship between the excreted choline and the other fractions of blood lipids.

Table 4 presents the ratios of the content of lipid phosphorus, cholesterol and total fat in the blood plasma to the amount of excreted

TABLE 4.—*Ratios Between Plasma Lipids and Choline in Diabetic Patients*

Patient	Cholesterol / Choline	Total Fat / Choline	Lipid Phosphorus / Choline
Patients with increased choline in urine*			
N. J.	55.47	183.1	1.96
R. M.	58.46	198.2	2.00
W. A. M.	45.35	126.9	1.27
A. K.	29.49	122.3	0.97
L. S.	32.47	159.7	1.48
H. V.	27.99	98.0	0.97
S. A. C.	37.48	124.4	1.35
Mean	40.96	143.8	1.43
Standard error	4.93	14.68	0.14
Standard error, per cent	12.02	10.20	9.93
Patients with normal choline in urine*			
L. E.	60.00	185.0	1.75
O. M. S.	54.80	177.6	1.86
M. H.	57.40	185.9	1.85
B. M.	108.40	300.4	2.94
A. B.	71.80	283.1	2.37
Mean	70.4	226.4	2.16
Standard error	8.80	29.31	0.22
Standard error, per cent	12.50	12.94	10.38

* Mean for three days.

choline. It will be seen that the ratio of lipid phosphorus : choline is the most stable of all, but this constancy is not appreciably different from the other ratios, as the standard errors of the mean show variations of only 9.93 to 12.94 per cent. This fact is not entirely in agreement with the concept that the relationship between choline and lipid phosphorus should be closer than the relationship between choline and other fractions of the blood lipids. How can this be explained?

It has already been mentioned in the comment on table 1 that the choline excretion of one subject shows great differences; for normal subjects it may show variations from some 0.1 mg. up to 5 mg. per day. These variations are dependent mainly on the amount of choline ingested

14. Schlegel, J. U.: Variations in Choline Content in Human Serum, Copenhagen, Ejnar Munksgaards Forlag, 1948.

with the food. The lipid phosphorus level is in this respect much more stable,[8a, c] and even after a fat meal an only moderate increase in the blood level is obtained.[12e] An additional reason why no agreement in the fall and rise of both is found rests on the fact that the relationship between choline in the urine and the phospholipids in the blood is too complicated to permit stable ratios to be established. Increased excretion of choline and increased phospholipid content in the blood are only the manifestation and the result of an impairment of various intersecting chemical processes connected with the fat metabolism, and it also stands to reason that little value can be attached to ratios taken from table 4, as it is hardly proper to make such direct comparisons. The relationship between the choline excretion and the phospholipid content of the plasma can therefore be established only by computation of significant differences, as shown in table 3. If over a period of several days one finds an increased daily choline output, it is certain that the phospholipids of the plasma will also be found to be above the normal range. At least the phenomenon that all the phospholipids contain choline provides further support for the evidence of a closer relationship between the compound and its constituents.

In the subjects examined by me, no relationship between the severity of the diabetes and the range of lipid phosphorus could be found (table 2). This confirms the finding presented in our previous paper,[8] viz., that there is no relationship between the high choline output and the excretion of ketone bodies. These observations agree with those of other authors, who found that diabetic lipemia is not directly connected with glycosuria and ketonuria.[15] It seems that insulin does not act directly on the choline metabolism or on a certain part of the fat metabolism which is connected with the function of choline. This hypothesis requires further confirmation and will be the subject of our next investigation.

SUMMARY AND CONCLUSIONS

The experimental work presented in this paper comprises a study on the relationship obtaining between the amounts of choline excreted in the urine and the content of cholesterol, total fat and lipid phosphorus in the blood plasma. It is shown that there is a relationship between the amount of choline excreted in the urine and the blood level of lipid fractions, mainly between choline and phospholipids, as an increased amount of excreted choline is always accompanied with an increased lipid phosphorus content in the blood, and vice versa.

No correlation was found to obtain between the high lipid phosphorus content of the blood and the severity of diabetic symptoms. Perhaps insulin does not act directly on the choline metabolism or on that aspect of fat metabolism connected with the function of the choline.

15. Bloor, W. R.: The Lipoids ("Fat") of the Blood in Diabetes, J. Biol. Chem. **26**:417, 1916. Blix, G.: Diabetic Lipemia, Acta med. Scandinav. **64**:142, 1926.

CHLORAMPHENICOL IN TREATMENT OF EBERTHELLA TYPHOSA G OSTEOMYELITIS

Report of a Case

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THIS CASE is reported because of the rarity of the type G typhoid bacillus, the infrequency of typhoid osteomyelitis in the United States and the promising status of chloramphenicol (chloromycetin®) in treatment of patients with typhoid infections.

REPORT OF CASE

History.—R. C. was a 27 year old man who first presented himself at the infirmary at Emory University in March 1948, because of recurrent attacks of pain in the right shoulder. During the war he had served as a Royal Air Force pilot and had been stationed in Eastern Canada, the United States, Great Britain, Spain, Gibraltar, France, Malta, French Morocco, Algeria, Tunisia, Cyprus, Palestine, Iraq, Iran, India, Ceylon, Saudi Arabia and Egypt. From July 1946 until the time of writing he had been residing in southeastern United States. He had received his first immunization against typhoid in 1940 and continued booster shōts at yearly intervals. It is surmised that he became infected while in the service, sometime between January 1945 and June 1946, when he was in areas in which typhoid, type G, was definitely endemic. This date is by no means certain; it is notable that during that time he was never ill with any sort of sickness that he could remember, except for several episodes of self-limited gastrointestinal upsets common among army personnel.

First Episode.—The first indication of any abnormality occurred in June 1947, when the patient experienced a dull, aching, boring sort of pain in the right shoulder with some radiation down the arm. This began rather suddenly after a game of softball. Limitation of motion of the arm was minimal. There were no systemic symptoms such as lassitude, malaise, fever or chills at that time. The difficulty was diagnosed as a "pulled shoulder" and symptomatic treatment instituted. During the next five weeks the pain gradually lessened and finally disappeared completely.

Second Episode.—In November, four months later, the pain in the right shoulder recurred without any apparent precipitating cause. It persisted for three weeks and seemed to be improved by the local application of heat.

Third Episode.—A third attack of pain in the right shoulder occurred in March 1948. Physical examination was noncontributory except for local findings of extreme discomfort from deep pressure over the right deltoid muscle. Although motion of the arm was not limited, the pain was intensified by strong abduction and backward rotation of the right arm. A roentgenogram made of the shoulder

Parke, Davis & Company supplied the drug used in this study.

From the Student Health Service, Emory University, and Emory University School of Medicine, Atlanta.

at that time showed a cystic swelling of the middle third of the humerus, with destruction of the medullary cavity and its replacement by tissues of homogeneous density which were fairly translucent and appeared to be loculated. There was scalloping of the inner cortex but no definite penetration of the cortex at any point. The distal end of this cystic area was well demarcated from the normal medullary bone, but the proximal end was not. The whole area of degeneration measured 10 cm. in length. Of a number of possible diagnoses that were entertained, chronic osteomyelitis seemed likeliest. On March 26 a subperiosteal abscess was aspirated and 2 cc. of bloody, purulent material obtained. This material revealed a pure culture of Eberthella typhosa. The organism was verified and further classified as Vi phage type G by the Georgia Department of Public Health.

Whereas the patient had had no systemic symptoms previously, after the aspiration more pain and cellulitis developed locally. He became moderately ill with malaise, fever and chills. No other symptoms developed. Repeated cultures of the stool and urine were negative for E. typhosa. Agglutination of E. typhosa H occurred at a dilution of 1:800, and that of E. typhosa O, at 1:80. Although the patient was given 2 Gm. of streptomycin and 6 Gm. of sulfadiazine per day, a sinus tract developed which drained thin serous fluid. This was sterile on repeated cultures. A low grade fever persisted, but all systemic symptoms disappeared. Roentgenograms made in July and August showed regression of the previous bone lesion, with residual fusiform dilatation of the midportion of the shaft of the right humerus.

Fourth Episode.—The patient remained well until November 22, when, without apparent cause, the pain in the right shoulder again caused him to be readmitted to the infirmary. The sinus tract was well healed, but its general area was tender and indurated. Although a roentgenogram showed no essential change from the previous one, it was supposed that a new subperiosteal or medullary abscess had developed. On November 23, the midportion of the right humerus was drilled with a Kirschner wire and the marrow cavity aspirated. No purulent material was obtained, and the aspirate was sterile on culture. The pain in the arm was accentuated by the manipulation, but no systemic symptoms developed. Laboratory data were as follows: There were 1,500 white blood cells, with 1 per cent band forms, 72 per cent segmented forms, 1 per cent eosinophils, 18 per cent lymphocytes and 8 per cent monocytes; the hemoglobin concentration was 11.5 Gm. per hundred cubic centimeters; the urinalysis was noncontributory, and agglutination of E. typhosa H in the Widal test occurred at a dilution of 1:40 and that of E. typhosa O at 1:800. Streptomycin, 1 Gm. per day, and sulfadiazine, 4 Gm. per day, were again administered. The patient was discharged from the infirmary after four days but continued the medication with sulfadiazine until November 27, when he was readmitted because of much increased pain in the arm and distressing generalized symptoms of fluctuating fever, nausea and vomiting and severe headache. It seemed apparent that the local infection had spread and was accompanied with intermittent bacteremia. The aforementioned medication was given and symptomatic treatment instituted. In two days the systemic symptoms subsided, and on the fifth hospital day the patient was feeling well except for mild pain in the right arm.

On December 15 a small area in the lateral aspect of the arm became increasingly fluctuant and finally ruptured spontaneously, draining a thin serous fluid.

On December 20 E. typhosa, Vi phage type G, was again isolated from a culture of this fluid. At about the same time repeated cultures of the blood, urine, stool and bile were negative for the same organism.

The patient's clinical condition remained stationary during that time. Except for an occasional elevation of temperature he was asymptomatic. The right upper arm remained swollen, indurated and somewhat tender.

On December 27 administration of chloramphenicol was begun, in doses of 0.25 Gm. orally four times a day. For the ten days prior to beginning therapy the patient had slight fever, the temperature ranging from 99.6 to 100.8 F., and constant tachycardia. The right upper arm was swollen and indurated. It was held fixed at the elbow at an angle of about 100 degrees, with limited flexion and no extension. Laboratory examination showed a trace of albumin in the urine. The white blood cell count was 9,200, with 73 per cent segmented forms, 4 per cent eosinophils, 1 per cent basophils and 22 per cent lymphocytes. The sedimentation rate was 49 mm. in one hour.

Roentgenograms made at the time of initiating treatment, on December 27, showed expansion of the medullary cavity of the midportion of the right humerus. There was now observed a soft periosteal reaction along the shaft, which completely encircled the midportion of the humerus. No new areas of destruction or sequestration of bone were observed.

The patient tolerated the drug well but the febrile course continued until the sixth day of treatment, when the temperature rose to 101 F. The arm became more tender and indurated. On the seventh day a pustule formed, overlying a sinus tract. At that time the dosage of chloramphenicol was increased to 0.5 Gm. four times a day, and 0.5 Gm. of streptomycin was injected into the sinus tract after aspiration of as much purulent material as could be obtained. The approach was made with a long no. 18 needle through healthy tissue. Despite the increase in the dosage of chloramphenicol, a sinus tract developed which drained a profuse amount of purulent material. The aspirate and the drained material were bacteriologically sterile. The fever gradually subsided, but drainage persisted throughout the fifteenth day, when the supply of chloramphenicol was exhausted. On the eighteenth day a new supply was obtained, and the patient was given 1.0 Gm. four times a day for forty-eight hours, after which he decreased the dose to 0.75 Gm. four times a day with minimal systemic reaction. He had slight nausea, and a macular, indiscrete rash developed on the forehead, chest and back. After dosage with 0.75 Gm. of the drug four times a day he became afebrile and the drainage gradually became less. With cessation of treatment on the twenty-fourth day he remained afebrile, and approximately ten days after treatment the sinus tract closed. The patient has been asymptomatic from then to the time of this report.

Recovery.—Roentgenograms taken one month after treatment revealed that the periosteal reaction previously described had a harder appearance and that there had been no appreciable increase in the periosteal deposit. There remained a tiny area of diminished density in the midportion of the expanded shaft.

Six months after treatment with chloramphenicol the patient was asymptomatic. Physical examination of the right upper arm revealed well healed sinus scars. Minimal induration of the soft tissues of the upper arm but no bony deformity or limitation of motion was determined. Roentgenograms of the humerus revealed less irregularity in the periosteal reaction previously described, and the margins of the shaft appeared smoother in outline. The area of decreased density in the midportion of the expanded shaft was less distinct than on previous examinations. Laboratory examinations revealed 5,152 white blood cells, with a normal differential count. The sedimentation rate was 8 mm. in one hour.

COMMENT

Vi phage type G was first identified by Craigie and Yen[1] in 1938, among cultures from Vancouver, Canada. Vi phage typing since that

1. Craigie, J., and Yen, C. H.: The Demonstration of Types of B. Typhosus by Means of Preparations of Type II Vi Phage, Canad. Pub. Health J. **29**:484, 1938.

time has revealed that this type occurs only rarely and seems to have a rather restricted geographic distribution. Among the 643 strains isolated from over 2,600 typhoid cultures typed by the Vi phage method by Felix [2] in England, there occurred only 2 type G cultures. These were obtained from a woman and her daughter who left Cairo, Egypt, on Sept. 25, 1945, and, shortly after their arrival in England, had an attack of typhoid due to Vi phage type G. Crocker [3] reported that among 495 South African strains of E. typhosa examined in Pretoria from 1942 to 1946 there occurred no type G strain. Wilson,[4] during the years 1943 to 1946, examined 286 strains from British Army laboratories in Northwestern Europe, Italy and the Middle East and found only 2 type G strains. In contrast, type G is common in Egypt. Boyd [5] reported that it was one of the commonest types occurring in prisoners of war with the British Army in Egypt during the period from December 1940 to February 1943. So far as is known, Vi phage type G has not previously been reported in the United States.

Typhoid-paratyphoid osteomyelitis is very rare, occurring as a complication of typhoid in only 0.87 per cent of cases.[6] We have been able to find only 27 cases reported in the literature. This is due partly to the fact that typhoid is becoming rare in the United States and partly to the fact that the long latent period between the fever and the development of osteomyelitis makes diagnosis difficult. The latent period may vary from months up to thirty-three years after the initial infection.[7] Apparently the organism finds the medulla favorable for survival over long periods, reactivation being caused by trauma or some unknown cause.

SUMMARY

A case of osteomyelitis due to E. typhosa Vi bacteriophage type G is reported. This is the first reported case of typhoid osteomyelitis in which chloramphenicol (chloromycetin®) has been employed. The response to treatment was not dramatic, but was followed eventually by cessation of all manifestations.

2. Felix, A.: Experiences with Typing of Typhoid Bacilli by Means of Vi-Bacteriophage, Brit. M. J. **1**:435, 1943.

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6. Veal, J. R.: Typhoid Osteomyelitis, Am. J. Surg. **43**:594, 1939. Murphy, J. B.: Bone and Joint Diseases Following Typhoid, Surg., Gynec. & Obst. **23**:119, 1916.

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CHEMICAL STRUCTURE OF SUBSTANCES EFFECTIVE IN TREATMENT OF PARKINSONISM

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AND
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IN 1882 GNAUCK[1] discovered the effectiveness of hyoscine in palliation of the symptoms of parkinsonism and opened the present era of medical therapy in that disease. In the next half century or more little was added besides the demonstration of similar action by the other alkaloids in the solanaceous group, atropine and hyoscyamine; extracts of the stramonium leaf,[2] and preparations of other plants containing the mydriatic alkaloids.[3] No one drug or combination of drugs has established its supremacy over the others because of the notoriously variable manifestations of the disease and pattern of response to therapy, which is constant only in the definite residue of obvious symptomatology following optimal medication.[4] In addition to certain drugs with limited application, such as amphetamine sulfate, occasionally useful in the postencephalitic syndrome, a wide assortment of compounds with nonspecific effect have been reported on, including barbiturates, salicylates,[5] and vitamins.

A preliminary report of this paper was read at the meeting of the Boston Chapter of the American Federation for Clinical Research, Boston, April 19, 1949.

From the Department of Neurology of the Harvard Medical School; the Neurological Unit of the Boston City Hospital, and the Tewksbury State Hospital and Infirmary.

1. Mendel, K.: Die Paralysis agitans, Berlin, S. Karger, 1911.

2. Stramonium preparations contain a mixture of alkaloids, especially atropine and hyoscyamine.

3. Price, J. C., and Merritt, H. H.: The Treatment of Parkinsonism: Results Obtained with Wine of Bulgarian Belladonna Alkaloids of U. S. P. Belladonna, J. A. M. A. **117**:335 (Aug. 2) 1941. Fabing, H. D., and Zeligs, M. A.: Treatment of the Postencephalitic Parkinson Syndrome, with Desiccated White Wine Extract of U. S. P. Belladonna Root, ibid. **117**:332 (Aug. 2) 1941.

4. Walshe, F. M. R.: Diseases of the Nervous System Described for Practitioners and Students, Baltimore, Williams & Wilkins Company, 1949.

5. Todd, A. T.: Treatment of Some Chronic and "Incurable" Diseases, ed. 2, Baltimore, Williams & Wilkins Company, 1947.

Because of the high percentage of undesirable side reactions (mainly loss of visual accommodation, decreased secretions and abnormal central nervous system stimulation) with the use of the solanaceous alkaloids, new compounds are always being sought. In recent years several lines of investigation have been followed: study of (1) the synthetic atropines, (2) other antispasmodics and (3) the antihistamine drugs and (4) other empiric drug-screening studies.

1. Of the synthetic atropines, trasentin,® dolantin,® parpanit,® pavatrin,® propivan® and syntropan® have been studied in Switzerland.[6] Parpanit® has been the most successful in clinical trials.[7] 2. Curare has been tried, but it has not merited wide use because loss of function following its administration parallels loss of tremor and rigidity.[8] Myanesin,® on the other hand, has been reported to achieve muscular relaxation and diminished tremor without debilitating loss of motor function,[9] but its value remains questionable.[10] 3. It was appreciated by early investigators that the anticholinergic activity of antihistamine substances might be of value in the therapy of parkinsonism. The use of such substances in the treatment of Parkinson's disease began with the treatment of 4 patients as a part of a routine

6. Domenjoz, R.: Parpanit, ein neues Therapeuticum bei Störungen der extrapyramidalen Motorik: Pharmakologie, Schweiz. med. Wchnschr. **76**:1282, 1946. Grünthal, E.: Ueber Parpanit, einen neuen, extrapyramidal-motorische Störungen beeinflussenden Stoff, ibid. **76**:1286, 1946. Hartmann, K.: Erfahrungen mit dem neuen Präparat "Parpanit" bei der Behandlung von Erkrankungen des extrapyramidalen motorischen Systems, ibid. **76**:1289, 1946; The Uses of Parpanit in Neurological Cases, Therap. Rev. **3**: (pt. 2) 162, 1947. Hartmann, K., and Monnier, M.: Significance of Electromyography and Electroencephalography in Diseases of Extrapyramidal-Motor System, Schweiz. med. Wchnschr. **78**:845, 1948.

7. (*a*) Schwab, R. S., and Leigh, D.: Parpanit in the Treatment of Parkinson's Disease, J. A. M. A. **139**:629 (March 5) 1949. (*b*) Dunham, W. F., and Edwards, C. H.: Parkinsonism Treated with Parpanit, Lancet **2**:724, 1948. (*c*) Frommel, E.: Pathogenesis and Treatment of Neurovegetative Disturbances in Parkinson's Disease, Praxis **37**:891, 1948. (*d*) Sciarra, D.; Carter, S., and Merritt, H. H.: Caramiphen Hydrochloride (Panparnit) in the Treatment of Diseases of the Basal Ganglions, J. A. M. A. **141**:1226 (Dec. 24) 1949.

8. Schlesinger, E. B.: Recent Advances in the Use of Curare in Clinical Practice, Bull. New York Acad. Med. **22**:520, 1946.

9. Berger, F. M., and Schwartz, P.: Oral Myanesin in the Treatment of Spastic and Hyperkinetic Disorders, J. A. M. A. **137**:772 (June 26) 1948. Gammon, G. D., and Churchill, J. A.: Effects of Myanesin Upon the Central Nervous System, Am. J. M. Sc. **217**:143, 1949. Schlesinger, E. B.; Drew, A. L., and Wood, B.: Clinical Studies in the Use of Myanesin, ibid. **4**:365, 1948.

10. Schwab, R. S.: Personal communication to the authors.

screening of benadryl® in many diseases.[11] Since then three antihistamine drugs have been given clinical trial in parkinsonism: Benadryl hydrochloride® [12] and thephorin® [13] have been reported on favorably and pyribenzamine® unfavorably.[12a] 4. The other drugs recently tried with success include artane® [14] and diparcol,® [15] the latter, however, evidencing many undesirable side reactions.

Historically the development of antihistamine drugs has followed two main structural lines: (1) the so-called "phenolic ethers," which are derivatives of ethanolamine and of which benadryl® is an example (fig. 1), and (2) the ethylenediamines, of which pyribenzamine® is an example (fig. 1). Most antihistamine drugs incorporate the same antihistaminic nucleus ($-CH_2-CH_2-N[CH_3]_2$), *R* in figure 2, and differ mainly in their side group and in the type of linkage to the side group. For example, in benadryl® (fig. 1) the side group is linked to *R* by an oxygen atom, whereas in pyribenzamine® it is by a nitrogen atom.

To date the therapeutic advances in parkinsonism have stood separately, and, despite similarity of clinical response to the several groups of effective drugs, no underlying correlation of chemical structure has

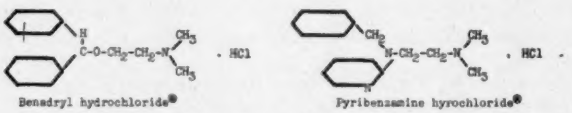

Fig. 1.—Examples of the two main types of antihistamine drugs.

been demonstrated. The present study was designed to determine what chemical configuration, present in the formulas of certain antihistamine drugs, may be essential to activity in the palliation of the symptoms of Parkinson's disease.

11. McGavack, T. H.; Elias, H., and Boyd, L. J.: Some Pharmacologic and Clinical Experiences with Dimethyl-Amino-Ethyl Benzhydryl Ether Hydrochloride, Am. J. M. Sc. **213**:418, 1947.

12. (*a*) Budnitz, J.: The Use of Benadryl in Parkinson's Disease, New England J. Med. **238**:874, 1948. (*b*) Ryan, G. M. S., and Wood, J. S.: Benadryl in the Treatment of Parkinsonism: Results in Forty Cases, Lancet **1**:258, 1949.

13. Blumberg, A. G., and Dattner, B.: Benadryl and Thephorin in the Treatment of Advanced Parkinsonism, read before the Section of Neurology and Psychiatry of the New York Academy of Medicine, March 8, 1949.

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15. Sigwald, J.; Bovet, D., and Dumont, G.: Le traitement de la maladie de Parkinson par le chlorhydrate de diéthylaminoéthyl-N-thiodiphénylamine (2987 R.P.): Premiers résultats, Rev. neurol. **78**:581, 1946. Frommel.[7c] Schwab.[10]

METHODS

In this investigation ten antihistamine substances have been given clinical trial: five ethanolamines—benadryl hydrochloride,® [16] L-01780,[17] S-111,[18] decapryn succi-

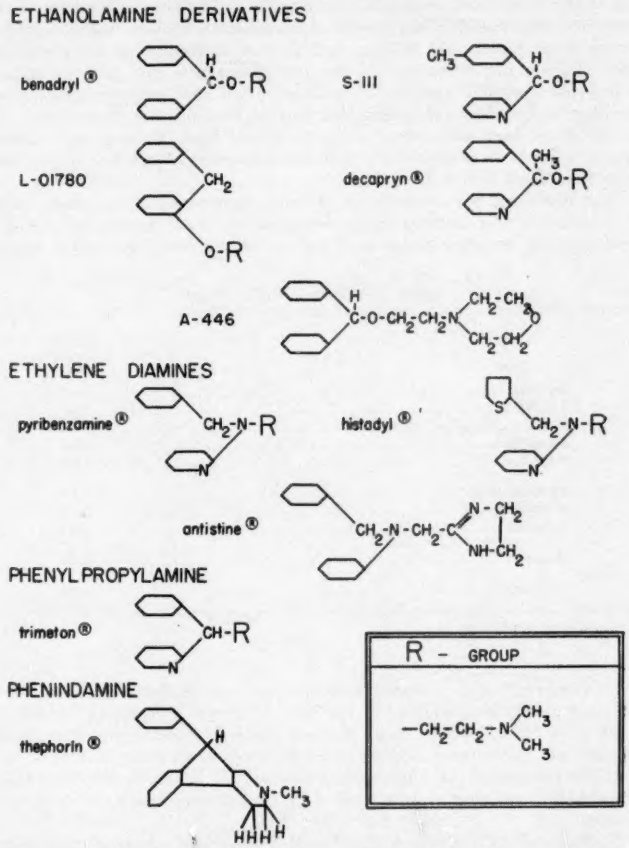

Fig. 2.—Structural formulas of the ten antihistamine drugs used in this investigation.

16. Benadryl® (beta-dimethylaminoethylbenzhydryl ether hydrochloride) and placebo were supplied by Parke, Davis & Company, Detroit.

17. L-01780 (investigational drug), an isomer of benadryl,® and placebo were supplied by Eli Lilly & Company, Indianapolis.

18. S-111 (investigational drug) (*p*-tolyl-2-pyridyl-*β*-dimethylaminoethyoxymethane) and placebo supplied by Schering Corporation, Bloomfield, N. J.

nate,® [19] and A-446 [20]; three ethylenediamines—pyribenzamine hydrochloride,® [21] histadyl hydrochloride® [22] and antistine hydrochloride® [23]; one phenylpropylamine—trimeton® [24]; and one phenindamine—thephorin® [25] (fig. 2).

An accumulated total of 62 patients with Parkinson's disease were observed over a period of eight months, during which time the ten antihistamine drugs listed were used (fig. 2). Thirty-seven of the patients were seen in the neurologic service of the Boston City Hospital, and 25 were hospitalized at the Tewksbury State Hospital and Infirmary. Of the total, 32 patients had paralysis agitans, 29 had postencephalitic Parkinson's disease and 1 had arteriosclerotic parkinsonism, according to the diagnostic criteria described by Denny-Brown.[26]

The drugs were administered orally in divided doses, three or four times a day, in amounts recommended for antihistaminic activity according to the total daily doses listed in table 1.

In a two month preliminary study of extrapyramidal disorders, using a group of 15 patients [27] not included in this series, it was found feasible to employ a rapid screening procedure of one week trial periods for testing the clinical efficacy

TABLE 1.—*Average Daily Doses*

Drug	Average Daily Dose, Gm.
Benadryl *....	0.300
L-01780....	0.300
S-111....	0.150
Decapryn *....	0.225
A-446....	0.300
Pyribenzamine *....	0.300
Histadyl *....	0.300
Antistine *....	0.300
Trimeton *....	0.150
Thephorin *....	0.150

* Registered trademark.

19. Decapryn® (2-[α-(dimethylaminoethyoxy)-α-methylbenzyl]pyridine succinate) and placebo were supplied by the Wm. S. Merrell Company, Cincinnati.

20. A-446 (investigational drug) (β-[1-morpholino]ethylbenzhydryl ether hydrochloride) and placebo were supplied by Parke, Davis & Company, Detroit.

21. Pyribenzamine® (N-alpha-pyridyl-N-benzyl-N′, N′-dimethylethylenediamine hydrochloride) and placebo were supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

22. Histadyl® (N,N-dimethyl-N′[2-thenyl]-N′[2-pyridyl] ethylenediamine hydrochloride) and placebo were supplied by Eli Lilly & Company, Indianapolis.

23. Antistine® (2-[N-phenyl-N-benzylaminomethyl]imidazoline hydrochloride) and placebo were supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

24. Trimeton® (1-phenyl-1-[2-pyridyl]-3-dimethylaminopropane) and placebo were supplied by Schering Corporation, Bloomfield, N. J.

25. Thephorin® (2-methyl-9-phenyl-2,3,4,9-tetrahydro-1-pyridindene hydrogen tartrate) was supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

26. Denny-Brown, D.: Diseases of the Basal Ganglia and Subthalamic Nuclei, Oxford Loose-Leaf Medicine, New York, Oxford University Press, 1945.

27. Ducey, J., and Gair, D. S.: Unpublished data.

of new preparations. This finding was in keeping with the fact that solanaceous alkaloid therapy generally elicits the greater part of its clinical response in less than one week. Schwab [7a] has also used one week trial periods.

At the initial examination the majority of the patients were weaned from their chronic medication by voluntary withdrawal or by the substitution of physically identical placebos for rabellon,® [28] bellabulgara,® [29] vinobel,® [30] benzedrine,® [31] dexe-

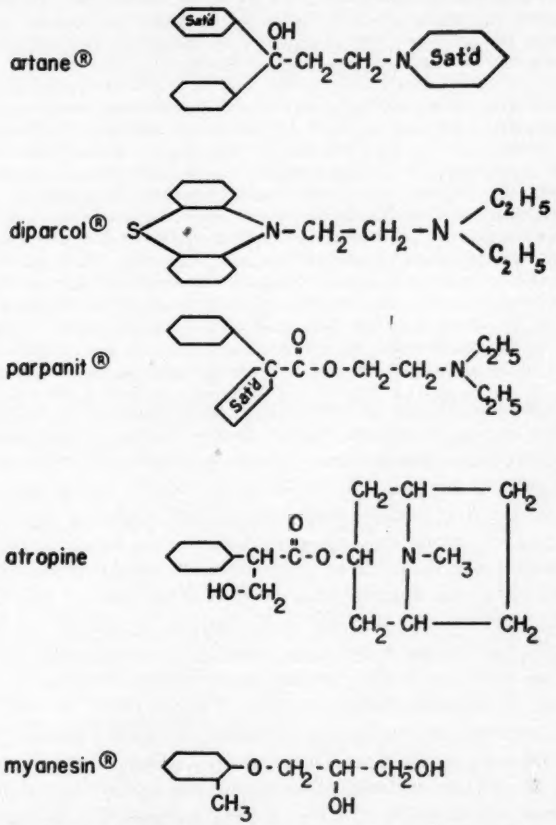

Fig. 3.—Structural formulas of atropine and drugs recently reported as effective in the treatment of parkinsonism.

28. Supplied by Sharp & Dohme, Inc., Glenolden, Pa.

29. Supplied by Lederle Laboratories, Division American Cyanamid Company, Pearl River, N. Y.

30. Supplied by the Wm. S. Merrell Company, Cincinnati.

31. Supplied by Smith, Kline & French, Philadelphia.

drine,[31] atropine sulfate[32] and hyoscine (scopolamine) hydrobromide[32] with ox bile salts added for bitterness and pills of dealkaloidalized stramonium leaf.[33] Each new drug was prescribed for at least one week, pills containing active principles being alternated with physically identical placebos for each of the ten compounds used.

For each patient a set of criteria were recorded in detail after each examination. These were quantitated on a 6 point scale. In reaching an assessment of the response to a therapeutic trial the changes in subjective well-being, general appearance, posture, facies, tremor, rigidity, performance tests and gait were the foundation for basic grading into three categories of change—namely, good, equivocal and failure. The other symptoms, such as dysarthria, dysphagia, sialorrhea, oculogyric crises, lassitude and insomnia, hyperhidrosis and greasy skin, compulsive actions and palilalia—varying from patient to patient—were given weight in this decision only when one or more of them was an obvious feature of the patient's symptomatology. Electromyography was not used because it cannot be quantitated to reveal the type of difference dealt with in this study any more accurately than does clinical evaluation.[34] An equivocal response was assigned to those patients in whom improvement was entirely subjective or objective changes were not accompanied with changes in subjective well-being. With one of these three grades of result—good, equivocal and failure—assigned in each test in each patient, the general effect of any given drug was arrived at by arithmetical summation of the number improved, the number of equivocal results and the number of failures. [NOTE: Hereafter the clinical effect of a drug in alleviating the symptoms of parkinsonism will be referred to as the AP effect of that drug.]

To demand miraculous improvement in order to assign a positive result to a drug would rule out all therapy extant in parkinsonism. The results here reported when positive represent merely reproducible responses to active principles reversed by placebos. The stigmas of parkinsonism elude precise quantitation, and it is largely on the reversal with placebos rather than on any absolute clinical estimation that evaluation can be made. Assay of the relative status of a patient with Parkinson's disease must be made by serial examination. Any of the patients seen for the first time under optimal therapeutic conditions with any effective drug in this study would represent no problem in diagnosis and would present a picture which might represent to many clinicians a therapeutic failure.

As with all other therapy in parkinsonism, the AP effect of antihistamine drugs manifests itself most clearly in subjective improvement, mainly in relief of stiffness. Objectively, the rigidity was easily the most improved condition, with tremor being mitigated but not markedly so. Other prominent changes were diminished sialorrhea, fewer oculogyric crises and relief from insomnia. Festination and hyperhidrosis in

32. Supplied by Parke, Davis & Company, Detroit.

33. Supplied by Davies, Rose & Company, Ltd., Boston.

34. Denny-Brown, D.: Interpretation of the Electromyogram, Arch. Neurol. & Psychiat. **61**:99 (Feb.) 1949.

some patients were remarkably improved. Subjective improvement so closely followed the picture of clinical improvement in this study that responses were graded equivocal or failures in its absence.

In some instances patients refused to continue experimentation because of the drastic placebo technic, and of a starting group of 62 patients 50 remained at the close of the study. Despite this predictable loss of cooperation, we feel that rigorous control with placebos is the only valid means at present to rule out major sources of error and to demonstrate positive objective results clearly.

The usual procedure was to have the patient stop all previous chronic medication with AP effect and to observe him after one week in the untreated state to establish a base line. Then benadryl® placebos were prescribed for one week, the patient was reexamined to determine any psychic benefit from the placebo and the active principle substituted. In the event of improvement with benadryl,® administration of the placebo was repeated. A relapse due to use of placebos was followed by the administration of the active principle again to control the relapse. After such a course on one drug, another drug was given and similarly alternated with placebos. Unless it controlled a placebo relapse, no drug was considered effective. Equivocal results were always checked by placebos; failures were not.

Thus, a typical patient with parkinsonism was first seen while he was receiving solanaceous alkaloids and was found to be moderately debilitated, with tremor and rigidity of arms and legs but able to walk. Chronic medication was stopped, and after three days he was bedridden and in need of constant care because of generalized rigidity. His sialorrhea was intense. He said that he had never felt worse and asked for some kind of medication; whereupon he was given placebos of a new drug. His condition remained unchanged, and he said at the end of a week that the drug was ineffective. Without his knowledge administration of the active principle was now instituted. After two days he was walking again, generally appeared as he did on initial examination and was without complaint. Placebos were substituted after one week, and within three days he was again totally incapacitated. Although mystified, he continued to take his prescribed medicine, which was changed back to the active principle after one week of placebo therapy. There was, as before, similar improvement. Analysis of the recorded data showed him to present a "good response." A second drug was given for one week, and the patient responded as to the placebo. This response was graded a failure. In this manner the ten antihistamine drugs reported on in this study were tested. All responses were as definite as described, although the degree of change was not in every case so dramatic.

Ten of the hospitalized patients had been taking benadryl® for six months. These were given placebos, followed again by the active principle one week later.

Some of the patients were unmanageable at first without their chronic medication and were subsequently weaned from it. Others were maintained on prior medication throughout. With these patients the regimen previously described was superimposed on the old drug schedule. Thus a total of 19 patients had the experimental regimen superimposed on chronic medication for a varying number of trials.

Since the determination of the most effective chemical configuration, rather than the dose for optimal effect of any one drug, was our primary consideration, little effort was made to adjust the dosage above recommended antihistaminic levels. On this regimen side effects were rarely encountered except in the case of thephorin® (discussed later). The complaint which occurred most frequently was that the placebo was a noxious drug. Since ineffective drugs produced changes similar to those of the placebos, no attempt was made to assign toxic qualities to them when administered immediately following an effective drug.

TABLE 2.—*Results: Good Responses to Antihistamine Therapy Given Alone and Combined with Administration of Solanaceous Alkaloids, and Total Equivocal Responses*

Drug	New Drug Alone		New Drug with Other Therapy		Totals		
	Trials	Good Responses	Trials	Good Responses	Total Trials	Total Good Responses	Total Equivocal Responses
Benadryl *.....	41	27	18	11	59	38	6
L-01780.....	38	4	11	3	49	7	13
S-111.....	29	1	2	0	31	1	10
Decapryn *.....	42	21	7	4	49	25	3
A-446.....	29	3	2	0	31	3	2
Pyribenzamine *...	34	6	5	1	39	7	7
Histadyl *.....	33	0	18	1	51	1	3
Antistine *.....	22	0	2	0	24	0	1
Trimeton *.....	35	21	19	12	54	33	5
Thephorin *.....	32	9	1	0	33	33	11

* Registered trademark.

RESULTS

The results are diagrammed in figure 4 and tabulated in table 2. Benadryl,® trimeton® and decapryn® stand out as effective in over 50 per cent of their trials, while the other seven drugs lag far behind.

Of the 10 patients receiving benadryl® for six months prior to substitution of placebos, six suffered typical relapse on placebo therapy followed by recovery on receipt of active principle.

Thirteen patients treated with thephorin® were maintained on the drug for more than one week because many showed a more delayed response to the medication than was evidenced by those treated with benadryl,® trimeton® or decapryn.® Three of these who had originally good response to thephorin® became worse without side reactions during continued administration of the drug, one after thirteen days, the second after twenty days and the third after twelve days. The majority of the patients showing side reactions to thephorin® suffered these to

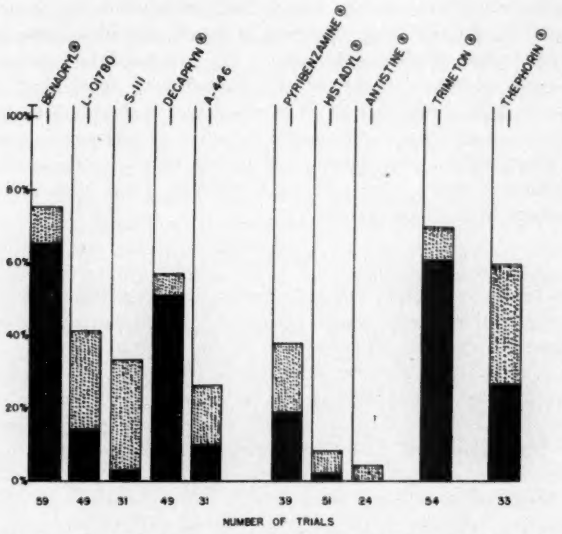

Fig. 4.—Results of the present investigation graphed in terms of per cent of trials of each drug. Solid blocks represent good responses; dotted blocks, equivocal responses.

a degree sufficient to have their response classified as equivocal or failure, thus lowering the recorded total good responses to a drug which actually demonstrates AP effect.

Drowsiness, the very common side effect of all antihistamine drugs, was noted in only 5 patients. In fact, many of the patients reported

TABLE 3.—*Incidence of Side Reactions*

Drug	Number of Side Reactions	Percentage of Trials of Each Drug
Benadryl *	2	4
L-01780	3	6
S-111	2	6
Decapryn *	2	4
A-446	1	3
Pyribenzamine *	1	2
Histadyl *	2	4
Antistine *	0	0
Trimeton *	2	4
Thephorin *	10	30

* Registered trademark.

increasing "pep" during the day as well as better sleeping at night when on an effective drug. Dryness of mouth, dizziness, insomnia and gastrointestinal disturbances were only occasionally encountered (table 3) except in those patients treated with thephorin,® among whom a high proportion of side reactions obtained. An explanation is not immediately apparent for the effect of thephorin® in causing side effects to the same extent as in normal persons whereas all other antihistamine drugs tested elicited a distinctly lower than anticipated percentage of such reactions.[35]

COMMENT

It has been reported that a synergism in AP effect exists between effective antihistamine drugs (benadryl,®[12a] thephorin®[13]) and the solanaceous alkaloids. Our results do not substantiate this concept. A number of trials of the ten drugs tested (table 2) were superimposed on solanaceous alkaloid therapy, and no significant difference in over-all response to effective drugs is notable between the groups tested with new drugs alone and those tested with the base line altered by other therapy. For example, benadryl® gave good results in 27 of 41 trials (66 per cent) alone and in 11 of 18 trials (61 per cent) given with other medication. Figures for trimeton® and for decapryn® are comparable.

In general, no striking differences in therapeutic activity were seen on replacing solanaceous drugs with an effective antihistamine. Where a new drug has been effective, it has been equally so whether it was added to or took the place of solanaceous alkaloid therapy.

We are aware of several pitfalls in this investigation which must be considered in evaluating the results. 1. In the event that physical dependence on effective drugs could develop within the brief period of administration, substitution of a placebo would cause a severe reaction by the patient which could readily be misinterpreted as a relapse and thus make the preceding period on active principle appear to show a good response. This could most readily be applied to the 10 patients given placebos after six months on benadryl,® but should their apparent relapses in this instance be due to physical dependence this would fail to explain subsequent good responses to other drugs unless a cross

35. Tolerance studies done on thephorin® by Boyd and co-workers (Boyd, L. J.; Weissberg, J., and McGavack, T. H.: Tolerance Studies of the Antihistamine Drug Thephorin,® New York State J. Med. **48**:1596, 1948) showed side effects in 24 per cent of 100 normal persons tested. Reactions included dryness of the mouth, insomnia, dizziness, weakness, nervousness and constipation. The amount of thephorin® given was 150 mg. a day in three divided doses, the same as used in the present study. In the same report both benadryl® and pyribenzamine® in the same doses were recorded as producing similar side effects in 61 per cent of the trials. The periods of study were analogous to those of the present series.

dependence existed. Since the majority of the trials were for sustained periods of only seven days, it is highly improbable that any physical dependence developed to account for the placebo relapses on this basis. Further, no withdrawal reactions to this group of drugs have been described in the literature. 2. Transient subjective improvements may be expected in many patients with parkinsonism following administration of a new nontoxic drug. In this series, however, the placebo technic, the use of many drugs which proved ineffective and the prolonged period of routine observation would appear to have neutralized such an effect adequately. 3. The possibility exists that the difference in effectiveness found for the drugs tested is related to varying factors of absorption and excretion, but this is most unlikely here, since all the drugs have been used as clinically effective antihistamine agents depending for their action on blood levels established by the same dosage ranges employed by us. 4. The inaccuracy that might be expected from weekly instead of daily observation has been overcome by the use of placebos for long periods, reports of relatives or medical personnel covering the interim between examinations and the long period of over-all observation. 5. That each drug was not followed for longer periods was because the projected scope of this experiment did not include determination of the limits of duration of effect of the drugs tested. Furthermore, the great majority of positive responses were reported as appearing on the second day, rarely later than the third. 6. An irremediable drawback of the placebo technic for testing long series of drugs is that there is an inevitable loss of some of those patients who suffer most by the administration of placebos and ineffective drugs. Since these need effective therapy most, they are the ones that will give clearcut positive responses and their loss from the roster of patients prevents effective drugs subsequently tested from demonstrating as high a percentage of positive results as those tested earlier in the series.

INTERPRETATION

Analysis of the formulas of the drugs used shows that the only features that are correlated with good clinical effect are (1) the antihistaminic nucleus, ($-CH_2-CH_2-N[CH_3]_2$), *R* in figure 2, which is identical in all but three of the drugs tested (A-446, thephorin® and antistine®), and (2) a symmetrically placed double ring side chain, present in trimeton,® decapryn® and benadryl® (fig. 2).

This general type side chain present in the three effective drugs is also found in the formulas of three ineffective drugs, S-111 (fig. 2), A-446 (fig. 2) and L-01780 (fig. 2), and when the published formula

is redrawn (fig. 5) in thephorin,® which gave contradictory results. These last four drugs, however, have differences in structure which must account for the poor responses to their administration.[36]

In the formulas of histadyl® (fig. 2), pyribenzamine® (fig. 2) and antistine® (fig. 2) the side chains are asymmetrically placed, and all three of these drugs are ineffective.[37]

It is apparent that no structural classification of antihistamine drugs includes all three of the effective drugs, for trimeton® is neither an ethanolamine, as are decapryn® and benadryl,® nor an ethylenediamine.

The structural formulas of artane,® diparcol® and parpanit,® all found to have an AP effect, may readily be compared with those of the three effective drugs (discussed in the preceding paragraph):

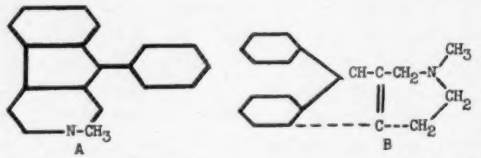

Fig. 5.—*A*, accepted structural formula of thephorin,® and *B*, the formula redrawn to show the antihistaminic nucleus and the R group.

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36. In S-111 one of the phenyl rings in the side chain is modified by a para-methyl group, while in A-446 the antihistaminic nucleus differs from that of the other drugs in having an ether ring system replacing the usual double methylation of the nitrogen atom. It should be noted that it is by this ether ring structure alone that A-446 is to be distinguished structurally from benadryl.® L-01780 is an isomer of benadryl® differing only in the position of attachment of the R group to the side chain. The resulting asymmetry of the chemical structure of L-01780 is correlated with failure to achieve results comparable to those observed with the administration of benadryl.® Thephorin® (a brand of phenindamine) is a derivative of pyridindene, and its accepted structural formula (fig. 5) reveals little similarity to the other drugs investigated. However, when the formula is redrawn (fig. 5), the R group and antihistaminic nucleus seen in the other formulas become evident and the drug now appears as a derivative of phenylpropylamine with the fundamental difference that two additional carbon atoms link the nucleus to the side chain in a complex ring structure. This must be charged with the responsibility for the variation in response to thephorin® (including the high incidence of side reactions) from that to trimeton,® which drug it would otherwise resemble.

37. Antistine® is the only drug in this series that elicited no positive response whatever, and the fact that both ends of its molecule differ from those of the effective drugs may account for this. The fact that all three ethylenediamines studied were failures does not rule out the possibility that an ethylenediamine with a symmetric side chain would be effective in parkinsonism. Indeed, Dipavcol® is such a compound.

Artane® structurally (fig. 3) contains the antihistaminic nucleus and the symmetric double ring side chain. The groups attached to its nitrogen atom differ from those in trimeton,® decapryn® and benadryl,® but apparently this does not modify adversely the effectiveness of the underlying configuration.[38]

Parpanit® (fig. 3) also is composed of a symmetrically placed two ring side chain linked to a nucleus differing from the usual antihistaminic nucleus in that it has a diethyl rather than a dimethylamine substitution.[38]

Diparcol® (fig. 3) is an ethylenediamine [37] generally conforming to the structural characteristics described in its nucleus and side chain but differing from the others in that a sulfur bridge joins the two rings of an otherwise typical side chain, thus creating a triple ring structure.[38]

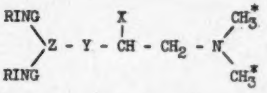

Fig. 6.—Skeleton formula for substances with parkinsonism-alleviating (AP) effect, except solanaceous alkaloids, benzedrine® and dexedrine® and myanesin.® C_2H_5 may replace CH_3 in the terminal valence bonds.[38]

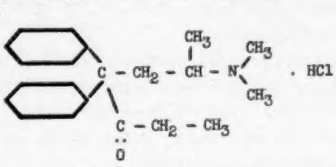

Fig. 7.—Structural formula of methadone.®

The present state of knowledge of the physical chemistry of atropine is too limited [39] to allow us to demonstrate common structural characteristics with the synthetic drugs discussed. Indeed, such characteristics may not exist (*e.g.*, meperidine [demerol®] is structurally unlike

38. The modification of the antihistaminic nucleus in artane® consists in a change in the substitution of the terminal valence bonds of the nitrogen atom. In artane® a saturated five carbon chain is added in ring fashion, and this is apparently without effect in altering AP potency. In A-446, however, interposing of an oxygen bridge in the middle of this four carbon saturated chain and creating an ether ring appears to abolish AP effect. Apparently the artane® ring acts much the same as the diethyl substitutions present in diparcol® and parpanit® in that it does not affect AP activity as compared with our results with dimethyl-substituted amine groups in this location in the molecules of effective preparations.

39. Three dimensional representation of atropine by Hirschfelder models is unsatisfactory according to present physicochemical concepts. Pfeiffer, C. C.: Nature and Spatial Relationship of the Prosthetic Chemical Groups Required for Maximal Muscarinic Action, Science **107**:94, 1948. Ing, H. R.: The Structure-Action Relationships of the Choline Group, Science **109**:264, 1949.

morphine). However it is evident that in atropine (fig. 3.) there is a tertiary nitrogen atom separated by two alkyl carbon atoms (in either of two directions) from a pivotal carbon atom, to which, in turn, are linked two complex chemical radicals. In this broad manner atropine may be said to resemble all the compounds here studied and those discussed, including, perforce, the effective drugs.

In figure 6 is diagrammed a skeleton formula fitting all drugs reported as successful in palliation of the symptomatology of Parkinson's disease, with the exception of the solanaceous alkaloids, benzedrine® and dexedrine,® and myanesin.® In the light of this discussion the essential portion of the formula is the tertiary ethyl amine conjugated symmetrically to a symmetric double ring radical.

Though the neurophysiologic implication is not known, it is of interest that this structure appears almost intact as part of the chemical configuration of methadone® [40] (fig. 7) which is one of the more potent central analgesics extant.

SUMMARY

Trials of ten antihistamine drugs in a series of 63 patients with Parkinson's disease are reported. The series covered an eight month period of observation and was controlled by the use of physically identical placebos of all drugs used.

Trimeton® and decapryn,® not previously reported in this connection, are recorded as being effective in palliation of symptomatology in 60 per cent and 51 per cent of trials respectively.

Benadryl,® previously reported as successful, is recorded as effective in this series in 64 per cent of its trials.

Thephorin,® previously reported as successful, elicited equivocal results with a high incidence of side effects and 28 per cent good results.

Pyribenzamine,® histadyl,® antistine,® A-446, L-01780 and S-111 elicited poor results.

Certain similarities in the chemical structures of the effective substances used and other reported effective medications are discussed, and a skeleton formula for an effective substance is described.

Under optimal medication in patients with Parkinson's disease there remains definite residue of obvious disease.

Parkinsonism is another example of unmasked physiologic processes that can be modified by chemical agents, though present knowledge is insufficient to allow complete delineation of the mechanisms involved.

22 Embankment Road.

40. 6-dimethyl-amino-4,4-diphenylheptanone-3 hydrochloride. VanDyke, H. B.: Synthetic Analgesic Drugs, editorial, Am. J. Med. **6**:681, 1949.

THE KIDNEY IN RELATION TO PROTEIN CATABOLISM

Effects of Abstention from Food and Fluid After Sudden Loss of Renal Tissue

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VARYING and contradictory prescriptions for consumption of food and fluid have been offered for treatment of patients in the initial stage of glomerular nephritis.[1] Basing his rationale on a particular concept of the pathogenesis of the disease, Volhard [1a] proposed that patients abstain from food and fluid for several days at the beginning of the disease. Such therapy has been widely used, and with apparent success. It has been used with the knowledge that it would not be the procedure to be recommended if a lessening of nitrogen retention were the sole consideration of treatment.

The experiments described in this paper were performed in the hope that, even though indirectly and by analogy, some indication might be obtained as to the probable effect of this treatment on the retention of nitrogen which always supervenes in some degree during the onset of glomerular nephritis. There is nothing in these results that can be taken as a direct contribution to the discussion of the theory behind this therapeutic method. All that has been done is to measure the effect of abstention from food and fluid on one aspect of a very complicated clinical situation, and that not on patients, but on rats in which retention of nitrogen was induced by the removal of three quarters of the total renal tissue. This technic was chosen because at the onset of glomerular nephritis there is a loss of effective renal tissue, a loss so sudden that it may be regarded as almost equivalent to that in partial nephrectomy. The effect was measured by comparing the

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From the Department of Medicine, Stanford University School of Medicine.

Miss H. J. Ureen, Mr. W. Lew, Mr. L. J. Poo and Mr. W. Wong gave technical assistance in this work.

1. (*a*) Volhard, F.: Die Behandlung der akuten Nephritis, in von Bergmann, G., and Staehlin, R.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1931, vol. 2, p. 1310. (*b*) Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, Lea & Febiger, 1939, pp. 479-489. (*c*) Addis, T.: Glomerular Nephritis: Diagnosis and Treatment, New York, The Macmillan Company, 1948, pp. 265-285.

rates of urea formation during successive four hour intervals after removal of renal tissue in animals fed a glucose solution with the rates in those to which water alone or nothing at all was given.

METHODS

The exact methods employed and a discussion of the measurement of urea formation have been described in detail elsewhere.[2] It should be noted that the formation of urea dealt with in this paper arises from the deamination of amino acids derived from the catabolism of body protein. For two days before operation the animals subsisted on a solution of 15 per cent glucose in 0.4 per cent sodium chloride solution and on vitamins of the B complex. This food was taken in large amounts and was calorically adequate so that in all animals a minimum nitrogen retention was approached, the serum urea concentration averaging 9.0 mg. per hundred cubic centimeters at the time of operation. The validity of the calculation of urea formation depends on the equality of the concentration of urea in all the cellular and extracellular water of the body.[3] Granted this equality, it follows that the total quantity of urea in the body is approximated by multiplying the serum urea concentration by the water content of the body. The rate of urea formation over successive four hour intervals was determined by adding the value for urea excreted during that time to the increase in the urea content of the body, as determined from the difference between estimates made at the beginning and at the end of the period. For the reasons already given,[2] this work was done on collections of pooled urine and serum made at the time each group of rats was killed. A total of thirty groups was used, comprising 263 female rats whose body weight at the start of the experiment was close to 150 Gm. At the end of the fourth, twelfth and twenty-fourth hours after excision of three quarters of the total renal tissue, twelve groups of rats were killed, three groups of 8 to 10 rats being killed after each period. These animals received no food and no water after operation. Another series of eighteen groups was given water but no food, and three groups were used for determinations at the end of the fourth, seventh, twelfth, sixteenth, twentieth and twenty-fourth postoperative hours. The results obtained under these two dietary conditions were compared with those obtained from the twenty-one groups already described,[2] that were given ad libitum amounts of 15 per cent glucose in 0.4 per cent sodium chloride solution after operation.

RESULTS

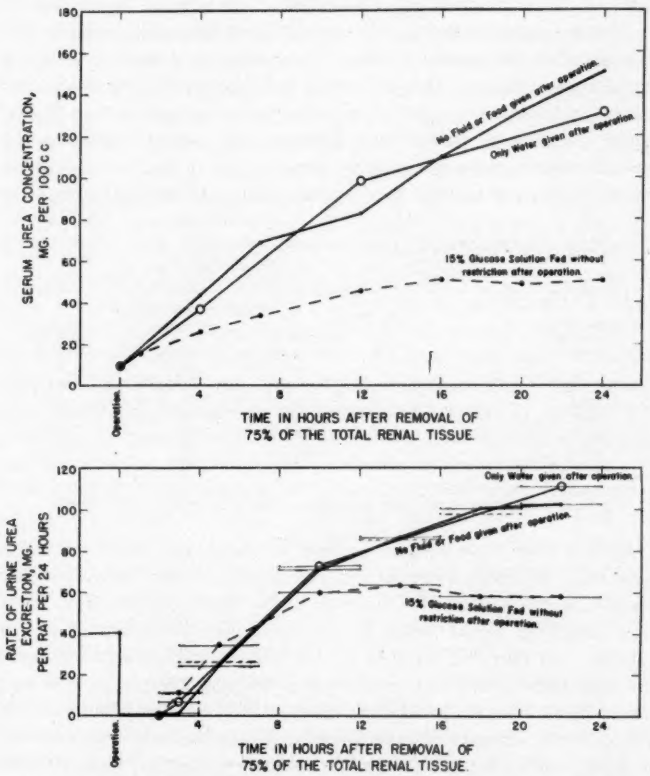
The increases in the urea concentration in the serum and in the rate of urea excretion of the starved animals relative to those of the glucose-fed controls are shown in the chart.

The difference in the urea concentrations in the serum was evident within four hours after operation and became progressively greater as time elapsed. At the end of the twenty-fourth postoperative hour the average urea concentration in the serum was 130 mg. per hundred

2. Persike, E. C., and Addis, T.: Increased Rate of Urea Formation Following Removal of Renal Tissue, Am. J. Physiol. **158**:149-156, 1949.

3. Marshall, E. K., Jr., and Davis, D. M.: Urea: Its Distribution in and Elimination from the Body, J. Biol. Chem. **18**:53-80, 1914.

cubic centimeters in the rats given only water and 150 mg. per hundred cubic centimeters in those deprived of water as well as food, as compared with the 52 mg. per hundred cubic centimeters found in the control rats fed the 15 per cent glucose solution. This difference in general is paralleled by that in the rates of urea excretion, but the


SERUM UREA CONCENTRATION, MG. PER 100 c c
No Fluid or Food given after operation.
Only Water given after operation.
15% Glucose Solution Fed without restriction after operation.
Operation.
TIME IN HOURS AFTER REMOVAL OF 75% OF THE TOTAL RENAL TISSUE.
RATE OF URINE UREA EXCRETION, MG. PER RAT PER 24 HOURS
Only Water given after operation.
No Fluid or Food given after operation.
15% Glucose Solution Fed without restriction after operation.
Operation.
TIME IN HOURS AFTER REMOVAL OF 75% OF THE TOTAL RENAL TISSUE.

Average urea concentrations in serum and average rates of urea excretion in urine. The excretory rates are plotted as midpoints, and the faint horizontal lines crossing the lines indicating rates of urea excretion represent the times during which the urine collections were made.

increase over the control level was proportionally less for the excretory rates than for the concentrations in the serum; the values for urea clearance thus were lower in the starved than in the fed animals.

The rates of urea formation calculated from these measurements are given in the table. The total amounts of urea formed during the twenty-four postoperative hours were 88 mg. per rat by the rats given the 15 per cent glucose solution, 182 mg. per rat by the animals given only water and 200 mg. per rat by the rats deprived of both food and water.

COMMENT

These results show that the rate of urea formation is more than doubled when no calories are taken after excision of three quarters of the total renal tissue. This increase is apparent within four hours after abstention from food. As far as water is concerned, it is not until twelve hours have passed that deprivation of water induces a still greater increase in the rate of urea formation. It should be remembered, however, that the animals used in this study had been drinking over

Rates of Urea Formation by Rats During First Twenty-Four Hours After Removal of 75 Per Cent of Total Renal Tissue

Period After Operation, Hours	Diet After Operation		
	15% Glucose Solution	Water Only	No Water or Food
0- 4	17.8*	27.4	32.8
4- 8	16.2	34.4	32.5
8-12	18.9	41.9	21.6
12-16	15.5	23.1	40.4
16-20	8.2	26.4	37.3
20-24	11.4	28.6	35.3
Total for 24 hours	88.0	181.8	199.9

* Values are in milligrams per 4 hour period per rat, corrected to 150 Gm. body weight.

a third of their body weight of water for forty-eight hours before the operation. When no water is given over periods longer than twenty-four hours after the loss of three quarters of the total renal tissue, a rise in urea formation occurs which quite overshadows the effect of lack of calories. Within the confines of the present experiments, however, the main effect arose from an absence of any external source of energy. The increase in urea formation observed in both experiments may be related to the necessity of meeting energy requirements by the catabolism of body constituents, and under the conditions observed here the additional urea formed must have originated from amino acids released by the breakdown of body protein.

The fact that within seven hours of the loss of 75 per cent of the total renal tissue the urea concentration in the serum of the starved rats was more than twice the concentration found in the serum of glucose-fed rats has a bearing on the question of whether patients should get no food at the onset of glomerular nephritis. It is probable that in man

as well as in rats the condition called uremia may be intensified by this deprivation. It is true that the substance that induces death in uremia is unknown, and that all that these studies have shown is that there is an increase in the concentration of urea, which is in itself not toxic, in the serum. But, since it is usual to see patients improve as the urea concentration in the serum falls and to find that as the urea concentration in the serum rises their condition deteriorates until they die, it is not unreasonable to suppose that the concentration of the lethal agent parallels, at least approximately, the urea concentration in the blood.

It would seem that no therapeutic plan which accentuates uremia should be followed unless there is good reason to believe that the disadvantage is more than compensated for by other advantages. To my knowledge, no good evidence that such compensation occurs has as yet been given. Such data as have yet been obtained from direct clinical observation regarding the efficacy of any plan of treatment in the initial stage of glomerular nephritis seem to be inconclusive. The lesions are extremely variable in intensity and extent. Each of them is, from the first, moving at its own rate toward repair of structure and reconstitution of function, and the present clinical methods do not enable us to observe, let alone measure, the effects of these many anomalies and their interrelationships. In the past, it has been shown that patients may improve under plans of treatment based on entirely contradictory principles. The notion of "vicarious excretion" led to purging, sweating and the induction of dehydration, but the idea of flushing out the kidneys with a large consumption of water also has been used. Experienced clinicians have reported good results from high and from low food protein consumption, and from diets which contained a variation of calories from zero to maximal amounts. From all this experience the only reasonable conclusions seem to be that it is difficult to defeat the reparative processes of nature and that the recovery of patients is not necessarily a validation of the methods of treatment used.

Since direct clinical evidence is so hard to evaluate in this situation, it would seem that the indirect method of inference from experimental work on animals is at present the safest guide. Nothing more than negative guidance can be hoped for from such experimental work in conditions so complex as those found at the onset of glomerular nephritis. Inferences from results such as those given in this paper perhaps may be applied with less reservation in the management of many other clinical situations associated with sudden renal failure. They have not been discussed in this paper because in these other disorders there is general agreement that any treatment which may induce an increase in uremia should be avoided.

SUMMARY

Under conditions in which urea is formed from the deamination of amino acids derived from the catabolism of body protein, 150 Gm. rats from which three quarters of the renal tissue had been removed formed the following amounts in twenty-four hours after operation: 88 mg. per rat when fed a 15 per cent glucose solution; 182 mg. when given only water, and 200 mg. when fed neither water nor food.

Certain negative inferences that may be derived from these results as to the current methods of treatment of patients in the initial stage of glomerular nephritis are discussed.

Progress in Internal Medicine

SYPHILIS

A Review of the Recent Literature

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MINERVA S. BUERK, M.D.
AND
WILLIAM T. FORD, M.D.
PHILADELPHIA

AFTER thirteen years of yeoman service, during which time thirteen excellent annual reviews of the recent literature on syphilis were produced,[1] the Johns Hopkins group, headed by Moore, has retired from this responsibility. We, the University of Pennsylvania group, have assumed the responsibility of continuing this annual effort with the hope that the results will be creditable.

Since July 1, 1948, hundreds of papers on syphilis have appeared in the world literature. They have dealt largely with penicillin therapy and serodiagnosis. The outstanding contribution of the year, in our opinion, was the appearance of Nelson and Mayer's study on immobilization of Treponema pallidum in vitro by antibody produced in syphilitic infection. This study opens new avenues for investigation of the biology of syphilis. This year also marks the twenty-fifth anniversary of the Kahn test, the first practical flocculation procedure for the serodiagnosis of syphilis. International problems of the venereal diseases, including syphilis, have been approached by the World Health Organization (WHO), whose efforts may yield outstanding contributions of value to venereal disease (syphilis) control.

From the standpoint of sources of pertinent literature on various aspects of syphilis, Stavisky's review [2] of the characteristics of pathogenic spirochetes and spirochetoses with special reference to the

From the University of Pennsylvania, Departments of Dermatology and Syphilology, School of Medicine, Donald M. Pillsbury, M.D., Professor, and Graduate School of Medicine, Herman Beerman, M.D., Chairman.

1. Tucker, H. A.; Mohr, C. F.; Hahn, R. D., and Moore, J. E.: Syphilis: A Review of the Recent Literature, Arch. Int. Med. **83**:87-115 (Jan.); 197-246 (Feb.) 1949.

2. Stavisky, A. B.: Characteristics of Pathogenic Spirochetes and Spirochetoses with Special Reference to the Mechanisms of Host Resistance, Bact. Rev. **12**:203-255 (Sept.) 1948.

mechanisms of host resistance, the symposium on syphilis edited by Eagle and Heller in the November 1948 issue of the *American Journal of Medicine,* Evan V. Thomas' text on syphilis [3] and Frazier and Li's monograph on racial variations in immunity to syphilis [4] are worthy of note. There is still a paucity of articles on syphilis in the foreign literature, but there are many indications that the prewar status of foreign scientific output will return.

In spite of the intensive study of penicillin dosage schedules for all phases of syphilis, the optimal treatment has not been determined. This is to some extent occasioned by the recent introduction of slowly absorbed penicillin products. Time for proper evaluation of these is too short, but they definitely promise a return to ambulatory syphilotherapy.

Aside from the problem of treatment, there are still many gaps in the knowledge of the biology of Treponema pallidum and of the course of syphilis. These deficiencies have been compiled by Wile as well as by Rosahn. Definite progress, however, has been reported on during this year in the fields of immunity, serology and epidemiology of syphilis.

UNSOLVED PROBLEMS IN THE BIOLOGY OF SYPHILIS

The biology of syphilis has received considerable and well deserved attention recently. In spite of the great advances in the general field, the following summations of the unknown by leaders in the specialty seem to emphasize the great lapses in our knowledge.

When he delivered the first William Allen Pusey Memorial Lecture of the Institute of Medicine of Chicago, Udo J. Wile [5] presented a stimulating, scholarly address with much food for thought. After discussing the unsolved problems in nature of the organism of syphilis and its life history, he presented the problems of animal experimentation. In considering the behavior of the organism in human syphilis, there is a peculiar selectivity of its affinity for certain structures (endothelial lining and musculature of blood vessels, bony structures and the parenchyma of various organs, such as the brain, liver and testes) and a paradoxic avoidance of other structures (parenchyma of the thyroid gland, pancreas, kidney and the ovary). While the smooth muscle of blood vessels is so often involved, that of the uterine body is never involved in either late or early syphilis. In like manner, structures lined with squamous epithelium are involved, while those lined with columnar epithelium are

3. Thomas, E. W.: Syphilis: Its Course and Management, New York, The Macmillan Company, 1949.

4. Frazier, C. N., and Li, H. C.: Racial Variations in Immunity to Syphilis: A Study of the Disease in the Chinese, White, and Negro Races, Chicago, The University of Chicago Press, 1948.

5. Wile, U. J.: Some Obscure and Paradoxic Problems of Syphilis, Arch. Dermat. & Syph. **57**:815-821 (May) 1948.

usually spared. In syphilis of the central nervous system there is again a rather extraordinary inconsistency in the reaction of comparable tissues to the invading organism. In dementia paralytica there are marked degenerative changes in the gray matter accompanied with a rich implantation of organisms, whereas in tabes dorsalis the degenerative changes are most prominent in the white matter and entirely unaccompanied with demonstrable organisms. The involvement of the second and third cranial nerves in neurosyphilis presents a baffling paradox. Anisocoria, the Argyll Robertson pupil and optic atrophy are too frequent concomitants of degeneration of the posterior columns and are relatively infrequent in dementia paralytica. Wile feels that habitus influences determination of type of parenchymatous neurosyphilis: tabes dorsalis in the asthenic type of person; conversely, but somewhat less strikingly, dementia paralytica in the pyknic types. Just as difficult to understand are the not infrequent involvement of the cortex, leading to dementia paralytica in congenital syphilis, and the extreme rarity of the tabetic syndrome in the same type of infection.

Rosahn [6] has also outlined those phases of the biology of syphilis which merit further investigation.

The Organism.—The predominant problem as far as the organism is concerned is the fact that the specific agent in its virulent form has never been cultured in artificial culture medium. To date, propagation of pathogenic treponemes has been possible only in the living organism. The unknown growth requirements of the pathogenic treponeme or the peculiarity of growth requirements remain a mystery. As a result, basic biologic studies have been seriously impeded. Are these missing links complex proteins, amino acids, enzymes, hormones or vitamins elaborated by the living host? Closely allied to the problem of growth requirements is the chemical constitution of the treponeme. Chemical fractionation of the organism should yield information as to the factors responsible for the tissue response in the living host. Systematic studies of the metabolism of the treponeme may provide information directly applicable to artificial cultivation. In the absence of artificial culture mediums, would it be possible to attain a sufficiently large volume of treponemes for chemical fractionation? If so, would difference in centrifugation destroy or alter the pathogenic properties of the resuspended organisms? Few attempts have been made to investigate the differential properties of the various treponeme strains and to classify them on the basis of common biologic characteristics. If the existence of classifiable strains could be demonstrated, many problems pertaining to human disease might be resolved. Another problem to be elucidated

6. Rosahn, P. D.: Unknowns in the Biology of Syphilitic Infection, Am. J. Med. **5**:631-640 (Nov.) 1948.

is the possibility of the existence of granular, invisible or transitional forms. Electron microscopy, filtration experiments, dark field motion pictures at high magnification and ultramicroscopy have all failed to indicate the existence of invisible or transitional forms. Final conclusions await the results of critical experiments.

The Host.—Syphilis is a natural disease of man but may be experimentally transferred to some laboratory animals, such as the mouse and the rabbit. In each of these hosts the disease reveals an ascending order of complexity. Mice fail to acquire lesions at the site of inoculation with emulsions of active rabbit syphilomas; yet the tissues of such mice are capable of producing treponeme-containing syphilomas when transferred back to rabbits. Furthermore, it has been proved that it requires several thousand organisms to infect a mouse; yet only one spirochete infects a rabbit. In addition, the destruction of the treponemes in the mouse takes place at a much greater rate than in the rabbit. Should the unknown inhibitory metabolic factors operative in the mouse be identified or isolated, it may be of some practical value when applied to the prevention of disease in man. In the mouse all tissues are nonreactive to the treponemes; in the rabbit only the testes, eye, skin and bone react with lesions, but in man no tissue is immune. If this tissue resistance in mice can be overcome, it may add some knowledge to the understanding of the human disease. The disease in rabbits more nearly approaches that of syphilis in human beings, but there are significant differences. Secondary lesions do not appear in the rabbit. What factors operate to inhibit the skin manifestations of syphilis? Cardiovascular syphilis, central nervous system syphilis and visceral gummatous lesions fail to develop in rabbits. Are there tissue resistance or inhibitory factors operating? Can the difference be attributed to the 2 degree increase in temperature or to the fact that the rabbit is relatively short lived? In the rabbit treponemes localize in lymphoid tissues and are readily recovered. This is not true of human beings with latent syphilis. Another difference between human and rabbit disease is the fact that in man primary or secondary lesions may be symptomatic. This situation apparently does not prevail in rabbits, as lesions always develop at the site of inoculation and secondary lesions never develop. There are innumerable unsolved laboratory and clinical problems in human syphilis. It is well known that not all persons exposed to infected patients acquire syphilis. Does infection depend on the number of organisms present at the source? What is the minimal number of treponemes necessary to produce infection, or is there some variation in individual susceptibility or does infectiousness vary with the strain of spirochete? Very little is known about natural or specific immunity to syphilis in man. It is quite evident that racial and sex factors play a

part. What influences are operating to localize infection in the vital organs in some persons and to inhibit specific lesions in others? Furthermore, little is known of the specific immunity. The traditional antibody-antigen reaction does not operate in syphilitic infections. Problems involving the serodiagnostic tests include the nature of reagin. Further study is needed to explain this mechanism of action. In addition, the method of infection in congenital syphilis requires elucidation. How does the pregnant state incite an invasion of the blood stream in a woman whose disease is of several years' duration? Why does the syphilitic pregnant woman escape development of syphilitic lesions in her own tissues? On what basis can the delay of tissue manifestations of syphilis in the fetus until the fifth month of gestation be explained? Arsenotherapy was introduced in 1910, but precise knowledge of the effectiveness of this form of treatment in preventing the late manifestations of the disease is still lacking. Routine autopsy surveys based on statistically adequate samples have yet to be pursued. Penicillin has revolutionized the therapy of syphilis and stimulated interest in the biology of syphilitic infection, but many problems remain unsolved. Some of these problems were presented in a discussion by Dr. J. E. Moore: In man, regardless of the treatment system employed, why is there a substantial residue of failure? What is the significance of low titer seroresistance in early syphilis after treatment with penicillin? Can criteria be devised to differentiate reinfection from relapse? What is the effect of penicillin on resting and rapidly dividing T. pallidum? Does prolonged seronegativity in man after penicililn therapy in early syphilis indicate cure? Why is an overwhelming massive infection in the fetus so readily curable when the penicillin concentration is lower than that in the maternal body? Is late syphilis curable by this drug? Are the good results in neurosyphilis permanent? Many of these basic problems can be solved only through physical and chemical research.

In an editorial introducing a series of articles which constitute a symposium on syphilis, Eagle and Heller[7] state that they cannot agree with the impression that the "last word" in the diagnosis and treatment of syphilis has been said. The demonstrated efficacy of penicillin offers hope that the incidence of new infections can be reduced to a "manageable minimum"; however, it seems unlikely that syphilis as an endemic disease can be totally eradicated. The epidemiologic problem remains to be solved. Present treatment programs are not definitive. The failure rate with penicillin in early syphilis is 5 to 15 per cent. The possibility of mass immunization must be explored. Details of the various papers in this symposium will be given at appropriate places in this review.

7. Eagle, H., and Heller, J. R.: The Symposium on Syphilis, editorial, Am. J. Med. **5**:629-630 (Nov.) 1948.

The interested reader is referred to previous summations of the unsolved problems in syphilology by Moore,[8] Beerman[9] and Stokes and Beerman.[10]

HISTORY

The period covered by this review has yielded little in the field of the history of syphilis. Marshall[11] discusses a thesis developed by Dr. J. Mottron entitled, "Rabelais et la vérole," which presents a survey of syphilis as it was understood in the first half of the sixteenth century.

J. Johnston Abraham,[12] in a freely readable style and with some excellent humor, discusses the treatment of syphilis from the epidemic during the so-called Siege of Naples in 1495 to the present time. Much space is devoted to mercury, the sole drug of value until 1835-1836, when potassium iodide was introduced. Arsenicals, malaria, bismuth, and finally penicillin are discussed in comparatively rapid fashion. Abraham concludes his remarks on penicillin with the statement, "Like Moses on Pisgah, perhaps, we may be looking into the Promised Land."

J. W. Eames,[13] of the Royal Army Medical Corps, prepared a lengthy, interesting report of definite historical value on the treatment of venereal diseases since 1898. Most of the report is devoted to syphilis, but gonorrhea, chancroid and nonspecific urethritis, too, are covered. Eames discusses in chronologic order treatment of syphilis with (1) hot baths, rubs with mercury ointment, mouthwash with alum solution and rubbing the gums with camphorated chalk; (2) pills of mercury with chalk; (3) mercurial inunctions for two years; (4) salvarsan (arsphenamine) and mercury; (5) neosalvarsan (neoarsphenamine); (6) bismuth, and, (7) penicillin. In each category complete details for carrying out the treatment are given.

Whether or not syphilis is alluded to in the books of the Old Testament is of more than passing interest to the venereologist, since the question is of importance not only in considering arguments for and against the Columbian theory of the American origin of the disease in Europe but also in the protracted dispute as to whether yaws and

8. Moore, J. E.: Unsolved Problems of Syphilology, Am. J. Syph., Gonor. & Ven. Dis. **23**:710-711 (Nov.) 1939.

9. Beerman, H.: Syphilis as a Field for Research by the Dermatologist, J. Invest. Dermat. **9**:113-124 (Sept.) 1947.

10. Stokes, J. H., and Beerman, H.: Some Problems in the Biology of the Syphilitic Infection, Am. J. M. Sc. **215**:461-469 (April) 1948.

11. Marshall, J.: Rabelais on Syphilis, Nature, London **162**:118 (July 17) 1948.

12. Abraham, J. J.: Some Account of the History of the Treatment of Syphilis, Brit. J. Ven. Dis. **24**:153-161 (Dec.) 1948.

13. Eames, J. W.: The Treatment of Venereal Diseases in the British Army, 1898-1948, J. Roy. Army M. Corps **90**:282-305 (June) 1948.

syphilis are or are not the same disease or modification of the same pathologic process. Willcox [14] discusses the problem with detailed documentation from the standpoint of idolatry; gonorrhea; syphilis; leprosy; syphilis, the "Egyptian disease"; syphilis, the plague of Moab; congenital syphilis; syphilis, Job's illness, and syphilis, David's illness. In summing up the biblical evidence the author questions whether there is enough to indicate that the disease was syphilis or, at any rate, that it represented the ravages of Treponema pallidum. If there is, the Columbian theory is weakened, though an open mind may still be permitted on the yaws-syphilis controversy. If the evidence is considered inconclusive and the argument too flimsy, the Columbian theory may be retained, though it must at the same time be admitted that syphilis, yaws, bejel, pinta and the like are separate diseases. This dispute is already centuries old, and in many respects it would be a great pity if someone did produce irrefutable evidence for either side and thus prevent any further such interesting speculation.

TREPONEMA PALLIDUM (AND OTHER TREPONEMES)

Davis [15] reviews the biologic aspects of the pathogenic members of the order Spirochaetales. He gives the classification of spirochetes as listed in "Bergey's Manual of Determinative Bacteriology." According to the new classification there are two families and three genera in each family.

FAMILY I. SPIROCHAETACEAE SWELLENGREBEL 1907
Genus I. **Spirochaeta** *Ehrenberg* 1833
Genus II. **Saprospira** *Gross* 1911
Genus III. **Cristispira** *Gross* 1910

FAMILY II. TREPONEMATACEAE SCHAUDINN 1905
Genus I. **Borrelia** *Swellengrebel* 1907
Genus II. **Treponema** *Schaudinn* 1905
Genus III. **Leptospira** *Noguchi* 1917

The family Treponemataceae is divided primarily on the basis of staining reactions and secondarily on conditions of growth. Species of the genus Borrelia stain easily with aniline dyes, whereas species of the genera Treponema and Leptospira stain with difficulty except with Giemsa's stain and silver impregnation. Species of the genus Treponema are strict anaerobes, whereas species of the genus Leptospira are aerobes.

Bergey's manual accepts eight species in the genus Treponema, including the causative agents of syphilis, yaws, pinta, spirochetosis of

14. Willcox, R. R.: Venereal Disease in the Bible, Brit. J. Ven. Dis. **25**:28-33 (March) 1949.
15. Davis, G. E.: The Spirochetes, Ann. Rev. Microbiol. **2**:305-334, 1948.

rabbits, the nonpathogenic Treponema microdentium from the normal oral cavity; Treponema genitalis from the human genitalia; Treponema calligyrum from smegma; and Treponema mucosum, which is reported as having pyogenic properties. As in the case of the relapsing fever borrelias and the leptospiras, there are adherents of the unity and plurality theories. In a recent expression for the unitarian interpretation Hudson places syphilis, yaws, bejel and pinta, as well as processes described under numerous other terms, in the category of treponematoses, all caused by one organism, T. pallidum. He considers the several "diseases" as different manifestations under varying conditions of climate, environment, hygiene, etc. The immunologic responses as expressed by the several serologic reactions are similar. The various reactions of the several treponemes in experimental animals and man give some information concerning the biologic relations of these organisms. In Treponema pertenue infections the rabbits had local and generalized lesions and positive serologic reactions and responded to treatment with neoarsphenamine. Individual lesions lacked the degree of induration observed in T. pallidum infections but, histologically, the lesions were quite similar to those of syphilis and yaws. It was concluded that the three organisms are closely related biologically but that the host-parasite reactions indicate substantial differences. Turner, McLeod and Updyke found that six months following the intratesticular inoculation of rabbits with T. pallidum, T. pertenue or Treponema cuniculi there was a considerable degree of cross immunity to each of these organisms. Padilha found that it was possible to obtain initial lesions of pinta in patients with yaws by inoculating them with Treponema carateum. However, the initial lesions appeared later and progressed more slowly than reported for normal persons. From the results of these experiments and similar experiments by other workers with yaws and syphilis, it is believed that there is a partial group immunity among these three treponematoses.

In an attempt to clarify the mechanisms of host resistance in spirochetal infections Stavisky [2] prepared an analysis and synthesis of the available data. This resulted in a bibliography of 239 pertinent books and articles, including several of his own earlier works. He found that many problems await study in this field and in order to stimulate research work prepared the accompanying table.

After an extensive and stimulating review of these topics the author concludes that the pathogenic spirochetes and spirochetoses conform very closely to the established principles of pathogenesis and host resistance in infectious diseases. The spirochetes may be divided into treponemas, borrelias and leptospiras. An essential similarity in the pathogenesis and host resistance in spirochetal infections is noted. The importance of antibody formation with lytic properties in acquired

resistance in spirochetoses is emphasized. Some characteristics of spirochetes, such as antigenic variability and capacity to produce relapses, may be related to their possession of the Wassermann type of lipid antigen. It is suggested that trypanosomes and trypanosomiases may be biologically related to spirochetes and spirochetoses. The author points to the spirochetes as a promising field of study for both the microbiologist and the immunologist.

The serologic relationships between five cultured strains of supposed T. pallidum (Noguchi, Kroó, Nichols, Reiter and Kazan) and

Outline of Some Problems in the Study of the Mechanisms of Host Resistance to Spirochetal Infections.

I. Parasitic factors

- *A. Morphology and life cycle.* Significance of coccoid bodies.
- *B. Electrokinetic potential.* Does it change in sign in spirochetes? If so, how? Importance of potential in relation to blood-brain barrier and phagocytosis.
- *C. Methods of accurately counting spirochetes.*
- *D. Cultivation of Borrelia and T. pallidum.* Growth factors and metabolism, especially as related to nonspecific inflammation in support of growth of T. pallidum.
- *E. Antigenicity and chemical composition.* Relation to pathogenesis of infection.
- *F. Toxic and aggressive substances.* Presence of hyaluronidase, fibrinolysin, leukocidin, coagulase.
- *G. Tissue tropisms.* Do Borrelia and Treponema exhibit a tropism for brain tissue?
- *H. Variation.* Is it induced or spontaneous? Does it occur in Leptospira and Treponema, and is it related to relapses and carrier state in these infections?

II. Host factors

- *A. Natural resistance.* What are the mechanisms of age and species resistance? Is antibody formation a crucial factor in natural resistance to leptospirosis?
- *B. Fixed cells.* How often does phagocytosis occur spontaneously without opsonins?
- *C. Antibodies.* Is complement necessary for lysis of spirochetes?
- *D. Hypersensitivity.* Is it necessary or helpful for operation of acquired resistance in syphilis?
- *E. Mechanism of carrier and relapse states.*
- *F. Infection-immunity.* Is it necessary for acquired resistance and, if so, how does it operate?

two strains of oral treponemes were studied by Eagle and Germuth [16] through the cultivation of the organisms and their properties, the method of immunization, the test for immunity to virulent treponemas and the agglutination and complement fixation tests. The data reported raise further doubts as to the propriety of identifying as T. pallidum the presently available cultured strains of treponemes, despite their supposed isolation from syphilitic lesions. None are pathogenic for rabbits, and all differ morphologically from the organisms as seen in lesions. All strains studied caused the formation of specific agglutinins and complement-fixing antibodies in high titer on injection into rabbits, but the rabbits did not have a positive Wassermann or flocculation

16. Eagle, H., and Germuth, F. G., Jr.: The Serologic Relationships Between Five Cultured Strains of Supposed T. Pallidum (Noguchi, Kroó, Nichols, Reiter and Kazan) and Two Strains of Mouth Treponemata, J. Immunol. **60**:223-239 (Oct.) 1948.

test reaction even after injection with a total of 11,000,000,000 to 20,000,000,000 organisms administered over a period of three to five weeks. In rabbits immunized with these cultured organisms and later inoculated with pathogenic T. pallidum a primary lesion developed after a normal incubation period. On the basis of their cross reactivity in agglutination and complement-fixation tests with rabbit antiserums, these five strains of cultivated treponemes fell into three distinct groups: (1) The Nichols and Noguchi strains were serologically indistinguishable; (2) the Reiter and Kazan strains exhibited cross reactivity but were not identical, and (3) the Kroó strain was serologically distinct from the other four. The Reiter strain was shown to be serologically almost identical with two strains of saprophytic oral treponemas. The data reported indicate that the five cultivated strains, all assumed to be T. pallidum, differ materially in their morphologic and antigenic properties from the pathogenic organism. The validity of their identification as T. pallidum is, therefore, questionable, according to authors.

Morphology of T. pallidum.—All students of the biology of syphilis are painfully aware of the shortcomings in regard to precise information on the morphology of T. pallidum. This is occasioned largely by the lack of means for propagation in culture of virulent strains of the organism and the resultant dependence for study on cultured avirulent T. pallidum-like organisms, organisms from the mouth or virulent organisms from experimentally induced infections in certain animals. The multiplicity of extraneous factors introduced by these circumstances causes wide variability in the results of reported studies. The relative limitations of the means of observation (dark field, electron microscope, etc.) introduce additional elements of confusion. In spite of these facts, we have noted definite progress in this field during the past year.

In 1926 Reiter reported successful cultivation of a spirochete isolated from a syphilitic lesion. No description of the organism was presented, but he considered it to be an acclimated avirulent variant of T. pallidum. Gelperin [17], of Johns Hopkins University, observed two distinct phases in the life cycle of the Reiter spirochete: (*a*) one in which spirochetes probably undergo transverse fission as the method of multiplication and which can be regularly produced by subculture every five to seven days in a medium containing 0.5 to 0.7 per cent agar and (*b*) one in which spherical "balloon-like" forms appear on the end of spirochetes when conditions in the medium become unfavorable for propagation. These "cysts" contain many small, round, translucent bodies which probably produce spirochetes when inoculated into fresh nutrient medium. The short actively motile forms of the spirochete predominate during the

17. Gelperin, A.: Morphology, Cultural Characteristics, and a Method for Mass Cultivation by the Reiter Spirochete, Am. J. Syph., Gonor. & Ven. Dis. **33**:101-113 (March) 1949.

period of logarithmic growth, and the longer spirochetes begin to predominate when the logarithmic period ceases. Brewer's thioglycolate medium, modified by omission of agar and reazurin, proved most satisfactory for maximum growth of the organism. Adequate anaerobiosis was achieved with a 0.1 per cent concentration of sodium thioglycollate. With the addition of 10 per cent by volume of heated serum and constant, gentle agitation by means of magnetic stirring, logarithmic growth was observed for two or three days, and multiplication did not cease until after the fifth or sixth day. Attempts to cultivate the organisms in mediums not containing serum were unsuccessful. Study of the rate of multiplication of Reiter spirochetes necessitated evaluation of various technics for measurements. Dark field examinations and turbidimetric, micro-Folin and Kjeldahl determinations were explored as alternate means of measuring growth. The various technics gave comparable results with the method of cultivation constant. The following quantitative measurements for Reiter spirochetes were recorded, (*a*) 1×10^9 organisms contained 58.5 mg. of nitrogen; and (*b*) 1×10^9 organisms, washed with saline solution and acetone and dried over phosphorus pentoxide weighed 0.8 mg. Under optimum conditions the division time of the Reiter spirochete was computed to be 8.8 hours for the first forty-eight hours. This is considerably less than the thirty to thirty-two hours reported for T. pallidum.

Hampp, Scott and Wycoff,[18] using the electron microscope, studied shadowed preparations of pure cultures of two strains of the small oral treponemes, four strains of Borrelia vincentii and the Nichols and Noguchi cultured strains of T. pallidum. They describe in minute detail the morphologic characteristics, filamentous and flagellar appendages and granules of various types. The article includes twenty excellent photographs with magnifications from 11,000 to 15,000 diameters, which well illustrate the features described.

Cumberland and Turner[19] describe a quantitative method for enumeration of spirochetes present in the rabbit testis based on dark field counts of emulsified rabbit testes. Normal and immune rabbits were inoculated intratesticularly with 10,000,000 spirochetes. Counts showed that 93 per cent of the treponemes inoculated into either normal or immune rabbit testes disappeared within two hours. Counts made on normal and on immune testes at one day after inoculation showed no significant difference, but by three days after inoculation the number of spirochetes found in immune testes was definitely lower than was

18. Hampp, E. G.; Scott, D. B., and Wycoff, R. W. G.: Morphologic Characteristics of Certain Cultured Strains of Oral Spirochetes and Treponema Pallidum as Revealed by the Electron Microscope, J. Bact. **56**:755-769 (Dec.) 1948.

19. Cumberland, M. C., and Turner, T. B.: The Rate of Multiplication of Treponema Pallidum in Normal and Immune Rabbits, Am. J. Syph., Gonor. & Ven. Dis. **33**:201-212 (May) 1949.

the number found in normal testes. The seven and eleven day counts showed very striking difference between normal and immune animals. Spirochetes in the normal testes became exceedingly numerous, whereas in the majority of the immune testes none or only a few organisms were found. The rate of multiplication of T. pallidum in the normal rabbit testis, as computed from counts of the number of spirochetes in the testis at various periods after their introduction, is approximately thirty-three hours per single division. This figure may be compared with that obtained by Magnuson and his associates, who calculated a division time of thirty hours from data on the incubation period of lesions on the back following intracutaneous introduction of graded inoculums. When spirochetes are introduced into the testes of immune rabbits there is a rapid initial loss, as in normal animals, after which the spirochetes may or may not increase in number, depending probably on the degree of immunity in the individual animal. When spirochetes do increase in number in the immune testis, the rate of increase is much less than that observed in the testes of normal rabbits. As a rule, the spirochetes slowly decrease in number and may disappear from the testis within one or two weeks after an initial brief period of multiplication.

CULTURE OF TREPONEMA PALLIDUM

After a brief review of the literature, Perry,[20] from the National Institute of Medical Science, London, describes a partially successful attempt to grow T. pallidum in tissue culture. The method employed is described in detail and is based on that described by Hawking (1945-1946) for the culture of Plasmodium gallinarum. The growth of testicular epithelium in vitro was confirmed, and successful cultures were obtained in most preparations in which the tests were taken from newly born rabbits. When the testes of young adult rabbits were used, cultures were also successful, although not so frequently as with newly born rabbits. The same technic was used for the in vitro growth of adult rabbit testis previously infected in vivo with T. pallidum. This method enabled some of the organisms to survive for periods up to ten days. Survival was apparently dependent on the presence of living testicular cell elements. Successful cultures were obtained in only a small proportion of preparations, however. The author hopes that further work may lead to improved results.

Eagle and Steinman[21] investigated the nutritional requirements of the cultivable Reiter strain with the hope that such data would offer

20. Perry, W. L. M.: The Cultivation of Treponema Pallidum in Tissue Culture, J. Path. & Bact. **60**:339-342 (April) 1948.

21. Eagle, H., and Steinman, H. G.: The Nutritional Requirements of Treponemata: I. Arginine, Acetic Acid, Sulfur-Containing Compounds, and Serum Albumin as Essential Growth-Promoting Factors for the Reiter Strain, J. Bact. **56**:163-176 (Aug.) 1948.

clues to the growth requirements of pathogenic T. pallidum. Four substances were identified as being essential for growth in the experiments conducted: (1) arginine, (2) acetic acid, (3) a series of compounds containing either free sulfhydryl groups or sulfur groups capable of being reduced to the sulfhydryl form and (4) crystalline serum albumin. The last was shown to be a substitute for the nondialyzable fraction of whole serum. The growth factors present in small amounts of yeast extract and casein which were necessary to provide satisfactory growth in the culture medium are yet to be identified. The basal medium employed in this investigation consisted of Brewer's thioglycolate medium and sterilized, filtered human or rabbit serum. A 9:1 mixture produced luxuriant growth, but in these experiments it was used in subminimal concentrations which alone did not support growth. A variety of substances were then assayed for their ability to permit multiplication of the organisms and were added quantitatively to the inadequate basal medium. If constituents of the basal medium would complicate the results, then these agents were omitted. Washed inoculum was used when the original culture fluid would complicate the interpretation of results. The tubes were incubated at 37 C. under anaerobic conditions. A direct microscopic count was made after four to six days. When arginine was added to the basal medium there was a striking effect on the growth curve, the number of organisms increasing in proportion to the concentration of arginine. The original inoculum contained 2,000,000 organisms per cubic centimeter, and after six days in the medium containing 1.6 cc. of tenth-molar arginine in 5 cc. the count was 114,000,000 organisms per cubic centimeter. Some forty-three other amino acids and related compounds were studied. These counts varied from 0 to 9,000,000 in the six day period. Dialyzed serum does not permit growth of Reiter's organism in thioglycolate-arginine medium, but the growth-promoting activity is restored when the serum dialysate is added. Dialyzed serum or dialysate used alone does not support growth. However, it was found that the factor or factors present in the dialyzable fraction of serum could be replaced by acetic acid. The beneficial action of sodium acetate was greater than that of ten homologous acids tested, and a quantitatively similar effect was obtained with fifteen of the thirty-seven related compounds tested. The growth curve obtained in the experiments employing acetic acid did not approach that of arginine, however. It was also found that the nondialyzable residue of serum could be replaced by bovine serum albumin. The growth-promoting activity of serum albumin increased as the concentration increased (up to 1 per cent). Sulfur-containing compounds are essential growth factors in the cultivation of Reiter treponemes. Cysteine proved to be efficacious in growth. The efficacy increased with increase in concentration of cysteine (up to 0.1 per cent

tested). Other sulfhydryl-containing compounds (glutathione, etc.) were active, but, in addition, several compounds in which sulfur was present were effective. Under the experimental conditions in this study arginine was found to be essential for the Reiter strain of treponeme. It appears that arginine as such may be an important part of the metabolic cycle, as similarly related compounds do not replace arginine. The fact that large amounts of arginine are required suggests that probably arginine is merely substituting for some more active compound as yet unidentified. Acetic acid was found to promote growth of the Reiter treponeme, but it was found that acetic acid could be replaced by a number of related compounds—acetaldehyde, etc. Acetic acid may also act as a substitute for the dialyzable components of serum. The growth-promoting properties of acetate and arginine are not evident unless there are present at least three other factors: (1) the unidentified factor(s) in the thioglycolate medium, (2) serum or serum albumin and (3) cysteine or some other sulfur-containing compound. Bovine serum albumin was found to be a growth-promoting factor and a substitute for the nondialyzable fraction of serum. As a result of this study five components have been identified as essential to the growth of Reiter treponemes under anerobic conditions: (1) arginine, (2) acetic acid, (3) any of the sulfur-containing substances, (4) crystalline serum albumin and (5) unidentified factor(s) in thioglycolate medium. Only three substances in thioglycolate medium could be contributing to growth: yeast extract, enzymatic casein digest and glucose. A study on these active compounds is in progress. A mixture of acetic acid, arginine, sulfur-containing compounds, crystalline serum albumin supplemented with minute amounts of enzymatic casein digest and glucose permits multiplication of the Reiter treponeme. The minimal effective concentrations of acetic acid and arginine are 0.0036 molar and 0.0005 molar respectively, but the growth rate increases progressively with increasing concentrations up to the highest concentration studied. The minimal effective concentration of the sulfhydryl compounds studied was 0.001 molar and that of serum albumin was greater than 0.1 per cent.

Survival of Treponema Pallidum in Vitro.—There has been continued interest in the factors affecting the survival of T. pallidum under varying conditions, a subject about which little seems to be known. Nelson and Steinman [22] made preliminary quantitative experiments to define various factors which influence the survival of T. pallidum in vitro. Using virulent T. pallidum (Nichols strain) obtained by emulsification with a Waring blendor® of rabbit testes in the early stage of

22. Nelson, R. A., Jr., and Steinman, H. G.: Factors Affecting Survival of Treponema Pallidum in Vitro, Proc. Soc. Exper. Biol. & Med. **68**:588 (July-Aug.) 1948.

acute syphilitic orchitis, it has been possible to compare the in vitro survival time of the organisms at a constant p_H in various gaseous atmospheres and at varying temperatures. The experimental work is described in detail. The authors found that under a given set of experimental conditions exposure to 5 per cent carbon dioxide had a definite prolonging effect on survival time. Survival was improved when temperatures below 37 C. were used. No indication of multiplication of the organisms has been noted.

In another study Nelson [23] found that in citrated human blood and plasma at 5 C. T. pallidum dies within seventy-two hours, though at —76 C. the organism will survive for years; in the chick embryo it apparently survives for only four hours. In order to study factors which affect survival, the author inoculated the virulent organisms of the Nichols strain into rabbits' testes and removed the latter within twenty-four to forty-eight hours after recognizable lesions had appeared. He used two technics for extraction of spirochetes from the rabbit tissues. His method is given in detail and consists essentially in washing of the tissue with cold isotonic saline solution, making a suspension in a "basal medium" consisting of crystalline bovine albumin, phosphate buffer, sodium thioglycolate, cysteine, glutathione, sodium pyruvate, a mixture of vitamins, sodium bicarbonate and sodium chloride, and agitating in a rotary shaker in an atmosphere of 1 or 5 per cent carbon dioxide followed by filtration to remove gross tissue and spermatozoa. Under the conditions of the experiment spirochetes did not multiply but did grow in length and tended to produce filamentous projections at one or both ends; half of the organisms survived for six to eight days and they retained their virulence. The main factors in maintaining viability of spirochetes are (1) maintenance of anaerobiosis by means of an atmosphere of 95 per cent nitrogen and 5 per cent carbon dioxide and addition of a reducing agent (sodium thioglycolate) to the basal medium; (2) albumin; (3) a p_H of 6.8 to 7.8; preferably 7.0; (4) inorganic phosphate; (5) a temperature of 30 C.; (6) tissue extract; (7) glutathione and cysteine; (8) sodium pyruvate. Various substances tended to reduce viability; for example, ascorbic acid and crystalline penicillin G caused immobility in the range from 0.001 to 0.1 microgram per cubic centimeter but did not cause more rapid immobilization beyond the latter figure. It is suggested that this method of prolonging the life of spirochetes may be a useful method of studying new antibiotics or chemotherapeutic agents.

Experimentally it has been shown by several investigators that if plasma or whole blood is seeded with virulent T. pallidum these organisms are no longer viable after the plasma or blood has been standing for

23. Nelson, R. A., Jr.: Factors Affecting the Survival of Treponema Pallidum in Vitro, Am. J. Hyg. **48**:120-132 (July) 1948.

forty-eight to seventy-two hours. Ravitch and his co-workers [24] in one year found a total of 487 blood donors with a positive serologic reaction for syphilis at the Johns Hopkins Hospital. Of these 1.2 per cent were white, and 13.5 per cent were Negro. The blood from these donors was discarded. The authors believe that there is already sufficient evidence to indicate that with the use of plasma in any form (since it is necessarily held for bacteriologic culture for one week before release) there is no danger of transfusion syphilis. They further state that as far as concerns the danger of transmission of syphilis, no serologic tests need be performed and no donor need be rejected in any plasma program. Therefore, there remains only the problem of whether the passive transfer of reagin produces a positive serologic reaction in the recipient. In order to clarify this phase of the problem, plasma of known relatively high reagin titer was injected into 16 persons with negative serologic reactions. The weights of these recipients varied from 39 to 104 Kg. Assuming that the plasma volume is 45 cc. per kilogram of body weight, then the computed plasma volumes for these 16 patients ranged from 1,755 to 4,680 cc. To these initial volumes there was added 500 cc. of plasma containing reagin. It can be calculated that the antibody was diluted in vivo approximately one fifth to one tenth of its original titer. After these 16 patients received transfusions of plasma with known amounts of reagin, their bloods were tested for reagin at twenty-four-hour intervals. In all 16 recipients, the acquired positive serologic reaction was reversed to negative by the twentieth day or before. It is pointed out that if the blood with positive reactions normally discarded by the blood bank were converted to plasma, together with all the blood with negative reactions processed into plasma, no pool would have more than one or two positive specimens in it. The resultant pool of plasma would have a very low titer of reagin and the duration of the persistence of the passively transferred reaction, if any were detectable at all, would be correspondingly brief. The authors conclude that the blood of donors with syphilis should be acceptable for use in any blood bank with a plasma program provided the material has been frozen and dried from the frozen state or stored at least four days in the refrigerator.

McLeod and Arnold [25] observed the effects of freezing at varying temperatures on the viability and virulence of the Nichols strain of T. pallidum. A 10 per cent suspension of spirochetes in rabbit serum was prepared aseptically from the testes of rabbits with early syphilitic orchitis. Dark field examinations of the suspensions were performed

24. Ravitch, M. M.; Farmer, T. W., and Davis, B.: Use of Blood Donors with Positive Serologic Tests for Syphilis, with a Note on the Disappearance of Passively Transferred Reagin, J. Clin. Investigation **28**:18-23 (Jan.) 1949.

25. McLeod, C., and Arnold, R. C.: Preservation and Inoculation Studies on Treponema Pallidum, J. Ven. Dis. Inform. **30**:104-107 (April) 1949.

before freezing. The suspensions were initially frozen at — 20 or — 78 C., and then each suspension was maintained at — 20 or — 78 C. for varying intervals, ranging from three, eight and sixteen weeks to one year. After the specified intervals the suspension was thawed at 37 C. and dark field examinations performed again, after which time each suspension was inoculated into a group of 5 rabbits. Inoculations were made into the right testicle and into the scrotal skin of the left testicle. As the lesions appeared, dark field examinations were again performed. All animals were observed for ninety days. Spirochetes which were frozen at — 20 and — 78 C. and maintained at — 78 C. remained viable for a period of one year. Spirochetes stored at — 20 C. remained viable for a period of four months. Survival was longer at this temperature if the initial freezing was carried out at — 20 C. rather than at — 78 C. Apparently exposure to a continuous storage temperature of — 20 C. is less harmful to the spirochete than a gradual change of temperature from — 78 to — 20 C. In rabbits inoculated with the frozen spirochetes lesions developed more frequently as a result of intradermal inoculation of the scrotum than as a result of intratesticular inoculation. It would seem that scrotal skin of the rabbit is a more favorable site than the body of the testicle for the survival of T. pallidum.

Spirochetes in Nonsyphilitic Diseases.—An important and interesting phenomenon is the finding of spirochetes in diseases other than syphilis. This always raises the question of the correctness of diagnosis of the presenting cutaneous disorder or the reliability of the means employed to demonstrate and identify the spiral organisms. Two new studies in this field appeared recently.

With the aid of a mercuric chloride solution for fixation of tissue, Lennhoff [26] developed a procedure for the demonstration of T. pallidum. The new spirochetes that he demonstrated are generally argentum-negative. This is probably the reason they escaped observation with the usual silver stains. He presents photomicrographs showing the spirochetes in histologic sections from patients with psoriasis, pustular psoriasis, seborrheic eczema, lichen ruber, pityriasis rosea, morbilli and zoster. In cooperation with Hollström, Lennhoff cultured spirochetes from patients with psoriasis, pityriasis rosea, lupus erythematosus and erythema multiforme.

Since Hollström,[27] in collaboration with Lennhoff, succeeded in culturing a short spirochete with characteristic movements from the lesions of pityriasis rosea, Hollström studied a series of patients with pityriasis

26. Lennhoff, C.: Spirochaetes in Aetiologically Obscure Diseases, Acta Dermat.-venereol. **28**:295-324, 1948.

27. Hollström, E.: The Treatment of Pityriasis Rosea with Spirochaeticides, Acta dermat.-venereol. **28**:325-329, 1948.

rosea treated exclusively with spirocheticides administered by injection. Thirty-nine patients were treated with iodobismuthite sodium (iodobismitol®) or bismuth subsalicylate and 7 with neoarsphenamine or oxophenarsine hydrochloride (mapharsen®). Each patient had three or four injections at intervals of three or four days. Complete or nearly complete absence of symptoms was achieved in an average of fifteen days in patients treated with the bismuth preparations and of twelve days in patients treated with the arsenical preparations. Most patients had the disease less than one week prior to institution of treatment. A Herxheimer reaction was observed in 4 cases.

EXPERIMENTAL SYPHILIS

There has been much activity in the field of experimental syphilis during the year covered by this review. Portions of this work are specifically discussed in a number of other sections, e. g., pharmacology of antisyphilitic drugs, immunity, T. pallidum, etc.

Heretofore studies on induction of a "resistant" strain of T. pallidum have yielded unconvincing results, and critical examination of the reports of workers claiming to have enhanced the resistance of this organism shows that the resistance is comparatively slight. In fact, it is more probable that the reported variation in the experimental syphilitic infection in rabbits is due to factors in the host rather than to an increased resistance of T. pallidum to arsenic. Since there is no clearcut clinical criterion by which "arsenic-resistant" strains may be identified, reliance must be placed on experimental syphilis in rabbits to identify a strain which has acquired "arsenic tolerance" or "resistance" or a strain that does not become arsenic resistants. Probey [28] studied this problem of inducing "arsenic resistance" in the Nichols strain of T. pallidum in experimental syphilis by subcurative treatment with neoarsphenamine. In controlled experiments spirochetes were not rendered "arsenic resistant" by the subcurative therapy schedule employed, when neoarsphenamine was given either in a large single dose or in multiple small doses. The variation between the curative dose of neoarsphenamine in the experimental infection with the "resistant" strain and the normal strain, 40 and 30 mg. per kilogram respectively, is within the limits expected in experimental infection.

In a previous study, Beerman and Severac [29] found that a strain of T. pallidum had been identified which in rabbits had sporadically exhibited

28. Probey, T. F.: Attempt to Produce an Arsenic-Resistant Strain of Spirochaeta Pallida in Experimental Syphilis, Pub. Health Rep. **63**:1654-1659 (Dec. 17) 1948.

29. Beerman, H., and Severac, M.: The Problem of Treatment Resistant Syphilis, J. Invest. Dermat. **5**:269-282 (Oct.) 1942.

evidence of refractoriness to arsenical treatment. As the strain became more adapted to the new host (rabbit), this refractoriness became quantitatively less. In spite of the suggestive evidence for the possible existence of treatment-resistant strains, the problem is far from settled. Probey's work, therefore, should be extended and larger numbers of rabbits used. It is rather interesting that the existence of true penicillin resistence of T. pallidum has not been established.

In a stimulating report Rosahn [30] reviews briefly some of the simpler terminology of nuclear physics, surveys the methods used in tracer studies and makes recommendations for the employment of radioactive tracer technics in the study of syphilis. As explained by the author, isotopes are different forms (of different atomic weights) of a given element, all of which react chemically in an identical manner. Radioactive isotopes have varying degrees of stability and a characteristic half-life, which is the length of time required for the radioisotope to deteriorate to half of its original activity, during which time alpha particles, beta rays or gamma rays are emitted. Use of a Geiger counter permits calculation of the amount or quantity of the radioactive isotope. Tagging an element means the preparation of a radioactive isotope which, because it emits rays, can be traced in the tissues of the organism, affording knowledge as to its utilization, distribution and excretion. Only a minute and harmless amount of the tagged isotope is required for tracing in the organism. Three technics for tracing tagged elements are: (1) the in vitro technic, in which the tissues, body fluids or excretions of the host are weighted and ashed and the amount of radioactivity determined by means of the Geiger counter; (2) the in vivo procedure, by which the localization and concentration of the administered tagged element are determined by placing the Geiger counter over various portions of the body, and (3) autoradiography, in which a slide with a thick layer of photographic emulsion is mounted with sectioned tissues and developed, revealing dark zones which indicate the precise localization of the radioactive isotope in the tissues.

In a consideration of the possible use of tracer technics in studies on syphilis, Rosahn notes that the tagging of treponemes is more difficult than the tagging of other organisms because pathogenic treponemes have not yet been grown in pure culture; successful tagging has so far been accomplished in vitro, however, by adding a salt containing radioactive phosphorus (P^{32}) to the culture medium. All efforts to tag the treponeme would be enhanced by a knowledge of the chemical constitution of the organism, to which end a systematic biochemical study of the organism is desirable to discover any rare or unusual elements which it

30. Rosahn, P. D.: Radioactive Tracer Techniques and Their Possible Application to Studies in Syphilis, Am. J. Syph., Gonor. & Ven. Dis. **32**:307-316 (July) 1948.

may contain, the addition of which elements in tracer doses to various culture mediums might yield information on the metabolism of the organisms.

With respect to the tagging of therapeutic agents, the metabolism of arsenic has been investigated with tracer quantities of the metal, and it has been observed that in man arsenic in this form does not pass from the blood into the spinal fluid in detectable amounts, the largest amounts being stored in skeletal muscles. It is possible that more precise information could be obtained on the metabolism of therapeutic agents containing arsenic by tagging neoarsphenamine and oxophenarsine hydrochloride with a radioactive isotope of arsenic (As^{74}), using both human and animal hosts. Similar studies could be pursued with penicillin when a properly tagged preparation becomes available, according to the author. Frequent failures of treatment may be accounted for if such studies show that antisyphilitic arsenicals do not concentrate in large amounts in the lymph nodes of the syphilitic human being. Rosahn suggests: (1) further study of the metabolism of the treponeme, employing tracer methods, to throw light on growth requirements and suitable culture mediums; (2) the use of radiophosphorus, radioarsenic, or other radioactive isotopes in attempts to tag the treponeme in vitro or in vivo, and (3) attempts to tag the distribution of therapeutic agents, such as the arsenicals and penicillin, in the tissues of the host in order to understand their localization in syphilitic lesions.

Rosahn and his group[31] studied the distribution of the infectious agent of syphilis in the organs of mice in order to determine whether the mouse could be substituted for the rabbit as the assay animal in experimental syphilis. One group of mice was inoculated subcutaneously with the Nichols strain of T. pallidum obtained from testicular syphilomas of the rabbit; the second group, via the intraperitoneal route. No lesions developed in any of the animals. In from twenty-seven to ninety days after subcutaneous inoculation, excised organs were subinoculated into rabbits as a test of infectivity. The lymph node material produced lesions in 100 per cent of the rabbits; the skin produced positive results in 80 per cent; the spleens, in 57 per cent; the blood, in 27 per cent, and the brain, in only 21 per cent. In the intraperitoneally inoculated group the lymph nodes produced positive transfer in 93 per cent; the blood in 77 per cent; the spleens, in 71 per cent; the skin, in 48 per cent, and the brain in only 31 per cent. Only the skin showed significant difference in the proportion of positive results in the two groups of mice. Of the subcutaneously inoculated group, 80 per cent had positive results as compared with 48 per cent with positive results in the intraperitoneally

31. Rosahn, P. D.; Gueft, B., and Rowe, C. L.: Experimental Mouse Syphilis: I. Organ Distribution of the Infectious Agent, Am. J. Syph., Gonor. & Ven. Dis. **32**:327-336 (July) 1948.

inoculated group. After either method of inoculation mouse lymph nodes were most frequently and mouse brain least frequently invaded. The frequency of invasion of mouse brain was greater after long periods of infection. Asymptomatic primary infection in the rabbit was not observed.

Frazier and his colleagues [32] observed the development of atheromatous lesions in the aorta and of xanthomatous deposits in the irises of rabbits fed on a high cholesterol diet. The rabbits were inoculated with the Nichols strain of T. pallidum. In 4 albino rabbits iritis developed at the site of xanthomatous deposits. No attempt was made to isolate T. pallidum from the iris or ciliary body. Control animals showed no evidence of iritis.

The relative therapeutic effectiveness of penicillin, oxophenarsine hydrochloride and tryparsamide in asymptomatic neurosyphilis in the white mouse was studied by Magnuson [33] in 116 animals with central nervous system syphilis established by intraperitoneal inoculation of 1,000,000 T. pallidum organisms three months before treatment. Results demonstrated that penicillin is an effective therapeutic agent but that neither tryparsamide nor oxophenarsine hydrochloride is curative in doses up to the maximum tolerated dose. Penicillin in doses of 16,000 units per kilogram or more was therapeutically effective in significant numbers of mice. The 50 per cent curative dose was approximately 14,000 units per kilogram; the 90 per cent curative dose, approximately 64,000 units. Tryparsamide in doses up to 2,400 mg. per kilogram and oxophenarsine hydrochloride in doses to 36 mg. per kilogram failed to cure significant numbers of mice. The maximum tolerated dose of tryparsamide was 2,500 mg. per kilogram; of oxophenarsine hydrochloride, 34 mg.

LUETIN TEST

For two years Rottmann [34] used the luetin skin test systematically on patients in his clinic. In a study of 520 patients with syphilis 77 had no clinical signs of syphilis; they had a noncontributory history, a negative serologic reaction and a negative spinal fluid. They were discovered to have syphilis after the employment of a second intracutaneous inoculation with luetin, which was then strongly positive, while the first skin test gave a negative result. In 17 of these 77 patients a positive serologic reaction developed after the second skin test, whereas they previously had always had a negative serologic reaction for syphilis. The luetin

32. Frazier, C. N.; Boyd, C. D.; Grunwaldt, E. B.; Barker, E., and Davis, M.: Phenomena of Disease in Rabbits Fed Cholesterol and Infected with Treponema Pallidum, Am. J. Syph., Gonor. & Ven. Dis. **32**:480-482 (Sept.) 1948.

33. Magnuson, H. J., and Rosenau, B.: The Treatment of Asymptomatic Neurosyphilis in the White Mouse, J. Invest. Dermat. **11**:435-441 (Dec.) 1948.

34. Rottmann, A.: Neue Erkentnisse in der Diagnostik der Lues, Wien. med. Wchnschr. **98**:225-231 (May 29) 1948.

used in the entire study was a preparation of killed T. pallidum from rabbit testis, made by the Serotherapeutic Institute in Vienna and used under the name of "Luotest." The technic of the test consists in the intracutaneous inoculation of 0.2 cc. of "luotest" into both forearms. Blood is taken for serologic tests at the same time. The test is read twenty-four, forty-eight and seventy-two hours later. A reaction is positive only when twenty-four hours after inoculation a sharply circumscribed 20 to 50 mm. in diameter, erysipelas-like, bright red infiltration appears at the site of inoculation. This infiltration is still clearly visible forty-eight hours after inoculation. If after forty-eight hours the infiltrate still increases in size and becomes a plaque, lasting several days, the reaction is called strongly positive. Five days later the same procedure is repeated and the results interpreted in the same way. Another eight days later more blood is obtained to note changes in the serologic reaction. The reaction to the first skin test was negative in practically all patients with syphilis (mostly tertiary or neurosyphilis) tested. However, the second skin test always yielded positive results. In 27 nonsyphilitic patients with neurologic disease used as controls the first skin test and second skin tests both yielded negative results. In several treated patients with syphilis and also in patients with clinically proved syphilis with negative serologic reactions Rottmann has observed the serologic reaction to become positive after the repeat skin test. If only the flocculation tests show positive results in these patients with previously negative reactions, the author believes that the body has become allergic to syphilitic antigen and gives an allergic response in the circulating blood to this antigen. When the Wassermann complement fixation test also becomes positive after the repeated intracutaneous skin test, the author believes that an active syphilitic focus is still present in the body. The luetin in some way has activated this focus and has induced it to produce antibodies against the syphilitic antigen. The author [35] believes that he has diagnosed several cases of congenital or hereditary syphilis in which there were no clinical or serologic signs of syphilis. Several of the patients had obscure neurologic signs and were discovered to have syphilis by a second skin test. Further investigation of the family history revealed syphilis in parents or grandparents. Also in several cases of obstetric difficulty in which no evidence of syphilis could be obtained, the author discovered a second positive skin reaction. Later, evidence of syphilis became apparent in the families of these patients. Rottmann believes that the "luotest," the repeat skin test and the phenomenon of reversal of the serologic reaction for syphilis after the second skin test are real diagnostic tests for this disease.

35. Rottmann, A.: Kritische Betrachtung der spätluetischen Herderscheinungen der erworbenen und angeborenen Lues und ihre Bedeutung für eine Luesübertragung bei der Bluttransfusion, Wien. med. Wchnschr. **98**:385-388 (Sept. 4) 1948.

IMMUNITY

In an article difficult to summarize adequately Magnuson [36] has detailed the pertinent experimental and clinical literature since Chesney's review in 1927. The more recent findings or concepts are as follows: Magnuson, Eagle and Fleischman found that the minimal infectious inoculum of the Nichols strain of T. pallidum on intratesticular inoculation was one spirochete. The minimal infectious inoculum by the intracutaneous route was somewhat higher, approximately five spirochetes producing infection in 50 per cent of the animals. The same authors found asymptomatic infection extremely rare in normal animals. Failure to manifest symptomatic disease nearly always meant that the animals had escaped infection. These findings may be attributed to the differences in the adaptability of the strains of spirochetes used or the rigidity with which experimental factors were controlled. Temperature is a factor in the development of symptomatic syphilis in rabbits; these animals must be kept at a temperature below 75 F. for symptomatic syphilis to develop. Local factors definitely play a part in resistance or susceptibility to infection. Wilson found that the incidence of primary syphilis among soldiers of the Canadian army was significantly higher in uncircumcised than in circumcised men. He attributes this difference to local resistance. Von Werssowetz found that 50 per cent of named contacts of primary and secondary syphilis apparently escaped infection. Probably the size of inoculum, local factors and systemic factors were responsible in overcoming the invading organisms. There is some clinical evidence that infection with either yaws or syphilis may protect against the other of the two diseases. Experimentally there is some evidence to suggest protection against heterologous organisms, but it is difficult to evaluate the degree of resistance. The mechanism of immunity is still little understood. There is some evidence that reagin bears little if any relation to immunity. Reinfection can occur while the patient has a serologic positive reaction; large amounts of reagin in animals did not protect from syphilitic infection; further, the titer bore no relation to quantitative immunity in reinoculated immune animals. Lastly, with the rapid treatment methods of the day, it becomes necessary to reevaluate the so-called relapse versus reinfection rates. It is entirely possible that many of the so-called relapses are in reality new infections.

Magnuson and Rosenau [37] present the first in a series of studies utilizing a quantitative approach to the immunity problem. It is based

36. Magnuson, H. J.: Current Concepts of Immunity in Syphilis, Am. J. Med. **5**:641-645 (Nov.) 1948.

37. Magnuson, H. J., and Rosenau, B. J.: The Rate of Development and Degree of Acquired Immunity in Experimental Syphilis, Am. J. Syph., Gonor. & Ven. Dis. **32**:418-436 (Sept.) 1948.

on the concept that immunity may be relative and that degrees of immunity may exist which will be adequate to deal with minimum inoculums but may be inadequate to overcome larger ones. The original infections were produced by uniform intratesticular or intracutaneous inoculations of the Nichols strain of T. pallidum and were terminated by treatment with penicillin or oxophenarsine hydrochloride (mapharsen®) three, six, twelve or twenty-four weeks after inoculation. The acquired immunity was challenged six weeks after therapy by the inoculation of quantitated numbers of the homologous strain of T. pallidum at the site of the original inoculation. Three types of response have been obtained on reinoculation. The first was symptomatic reinfection in which a lesion showing spirochetes on dark field examination developed at the site of reinoculation. The second was asymptomatic reinfection in which no lesion developed at the site of inoculation, but the animal had been infected as shown by lymph node transfer. The third was immunity in which no lesion developed and the animal was not infected as shown by lymph node transfer. As the duration of the immunizing infection increased from three to twenty-four weeks, there was a progressive increase in the number of T. pallidum organisms required to produce the symptomatic and asymptomatic responses. Starting from a small but demonstrable immunity at three weeks, the immunity increased to such a degree that by the twenty-fourth week animals were partially protected (asymptomatic reinfection) against 10 minimal infectious inoculums. Two hundred thousand minimal infectious inoculums were required to produce symptomatic reinfection in 50 per cent of these animals and immunity in the remainder. There was little difference between the immunity of animals treated with oxophenarsine hydrochloride and that of animals treated with penicillin. The authors discuss the pathogenesis of asymptomatic infection (which is a rarity in the infected but untreated rabbit) and assume that a few treponemes have evaded the local barriers and have reached the lymph nodes. They regard the ultimate fate of treponemes causing these asymptomatic infections and their effect on the host as of the utmost importance and regret that the course of experimenal syphilis in the rabbit does not provide an answer to the problem. In attempting to apply this work to the course of syphilis in man, Magnuson and Rosenau consider that in patients treated early "reinfection" will tend to be of symptomatic type, whereas in those not treated until the secondary stage there will on reinfection be more likelihood of development of an infection of asymptomatic type, evident perhaps only as a serologic relapse.

Frazier and Li [4] wrote a monograph based on an enormous experience with 16,845 consecutive cases of syphilis in the Chinese, white and Negro races, dealing with the fundamental problem of immunity in syphilis. Scattered studies of race and sex differences in the

frequency with which late serious manifestations of syphilis occur have been made. In many instances, the studies have been based on such highly selected small groups of patients as to make the conclusions uncertain or impressionistic. Frazier and Li submit evidence, based on twenty-five years' study, that syphilis in both Chinese males and females tends to become latent in a much higher portion of patients than in white and Negro males and females. The Chinese male has nearly as good a chance that the disease will proceed to latency as does the white female, in whom the infection is most benign for reasons unknown. Early in the disease acute periostitis and acute meningitis tend to develop much more frequently in the Chinese male than in the white male. In spite of the tendency toward meningeal involvement early in the disease, tertiary localization in Chinese was preponderantly extraneural. The authors consider various backgrounds which might contribute to the benign course in the Chinese people, without coming to any definite conclusions. They do not subscribe to the idea that special strains of spirochetes may be neurotropic.

Antibodies in Syphilis.—Turner, Kluth, McLeod and Winsor [38] present the experimental method and results of so-called protection tests using serum from human beings in place of rabbit serum previously reported by Turner.[39] With their technic, serums from patients with secondary, latent or tertiary syphilis exerted a definite inhibitory effect on the development of syphilitic lesions in rabbits as compared with serums from nonsyphilitic patients. Serums from patients with primary syphilis in general behaved more like the serums from nonsyphilitic persons. They concluded that "persons with secondary, latent and tertiary syphilis as a group possess humoral antibodies which kill, or at least inhibit, the multiplication of *Treponema pallidum,* and that these protective antibodies probably play a significant role in acquired immunity to the disease."

Normal and abnormal cerebrospinal fluids were examined by Roemer [40] for evidence of spirochetal agglutinins. Since agglutinins are thought to be formed in the cerebrospinal fluid spaces, one would expect to find specific agglutinins in the fluid. The antigen employed in these agglutinations was "palligen" (prepared according to the method of Gaethgens, 1927), an antigen which has been used in serodiagnostic complement fixation tests. "Palligen" consists of a suspension of killed

38. Turner, T. B.; Kluth, F. C.; McLeod, C., and Winsor, C. P.: Protective Antibodies in the Serum of Syphilitic Patients, Am. J. Hyg. **48**:173-181 (Sept.) 1948.

39. Turner, T. B.: Protective Antibodies in the Serum of Syphilitic Rabbits, J. Exper. Med. **69**:867-890 (June) 1939.

40. Roemer, G. B.: Spirochätenagglutinine im Liquor cerebrospinalis bei Syphilis des Zentralnervensystems, Klin. Wchnschr. **26**:561-564 (Sept. 15) 1948.

spirochetes in a phenolized isotonic sodium chloride solution. The test used was the tube agglutination technic. Macroscopic flocculation was considered a strongly positive reaction. If the test produced a slightly ground glass appearance it was considered a weak reaction. In a negative reaction there was no evidence of agglutination. A control series of tests were run simultaneously according to the Meinicke clarification and Wassermann technics. In a series of 500 patients on whom examinations of the cerebrospinal fluid were done, 40 had some form of neurosyphilis. Of the total 500 fluids, 461 gave negative agglutination reactions to the Meinicke and "palligen" tests and only 1 gave a doubtful reaction to the Wassermann test; 33 gave positive reactions to the Meinicke and "palligen" tests, whereas 22 gave positive reactions to the Wassermann test. In only 1 case did the reactions to the Meinicke and the "palligen" tests disagree. In all cases in which the Wassermann reaction was positive, the "palligen" agglutination reaction was also positive. With only 1 exception, in all cases in which the Wassermann and the Meinicke reactions were negative, the "palligen" reaction was also negative; in the exceptional case the "palligen" reaction was positive. The author states that there is more need for sensitivity and specificity of serologic testing, and the results of tests with "palligen" antigen are promising. The spirochetal agglutination test is quick and simple, and only small amounts of fluid are needed. The test is a true antigen-antibody reaction and, therefore, unlike complement fixation or clearing reaction tests. It can be performed by the bedside, and a highly equipped laboratory is unnecessary. A negative and positive control should always be run simultaneously. It is also necessary to corroborate Roemer's results.

The outstanding study of the year, we believe, is that of Nelson and Mayer [41] dealing with immobilization of T. pallidum in vitro by antibody produced in syphilitic infection. Nelson and Mayer have experimentally demonstrated a heretofore undetected antibody in the serum of syphilitic animals and human beings. This antibody has been designated as an immobilizing antibody, and, as the name indicates, it has the ability to immobilize virulent T. pallidum organisms. The present serologic tests for syphilis are carried out with lipid antigens of mammalian tissues and serve to detect syphilitic infection with a high degree of specificity. These tests, however, are not an index of immunity. This is especially the case in latent syphilis when the titer of reagin decreases while the disease progresses. The authors are of the opinion that the immobilizing antibody may be related to the immunologic processes. The purpose of this detailed report is (1) to demonstrate the immobilizing activity due to a specific antibody in the presence of complement and to show that

41. Nelson, R. A., and Mayer, M. M.: Immobilization of Treponema Pallidum in Vitro by Antibody Produced in Syphilitic Infection, J. Exper Med. **89**:369-393 (April) 1949.

immobilized spirochetes are noninfectious or dead; (2) to study factors that affect the measurement of the immobilizing antibody in the serums of syphilitic persons; (3) to demonstrate the presence of antibody in serums from animals and human beings in various stages of syphilitic infection, and (4) to differentiate the immobilizing antibody from reagin.

The extraction and maintenance of virulent T. pallidum from rabbit syphilomas was described by Nelson in 1948. The extracted treponemes are separated from tissue debris under special atmospheric conditions and filtered, and the filtrate examined with dark field illumination to ascertain the number of spirochetes per high power field. Two spirochetes per field are equivalent to 10^7 organisms per cubic centimeter. Should the number exceed this, the filtrate is diluted with a special complex medium [23] to the desired (10^7) concentration. The percentage of motile organisms is determined by examining 50 successive treponemes in fields selected at random. Usually 90 to 98 per cent exhibit active motility. Counts of 100 or more organisms may be more precise, but the time consumed for such a procedure would subject the organisms to unfavorable conditions and lead to nonspecific loss of motility. When spirochetes are suspended in the complex fluid medium, they are extremely active. There are no intermediate phases of motility resulting from the action of antibody and complement, i. e., sluggisly motile forms. The organisms are either fully active or completely nonmotile. In the tests described the immobilized spirochetes are not lysed nor do they agglutinate. Accurate determination of the total organisms present and percentage of motility can be made because repeated counting of the same motile treponeme in adjacent fields is unlikely in view of lack of translational movements of the spirochete. The complement used in the immobilization antibody tests is obtained from the guinea pig. (Tests showed that guinea pig serum diluted 1:20 gives maximum results.) Five groups of serums were used for testing of the immobilizing antibody activity. These were obtained from (1) nonsyphilitic rabbits; (2) untreated syphilitic rabbits inoculated with Nichols strain with infection from three to nine months; (3) serums of patients with primary and with secondary syphilis confirmed by dark field examination; (4) serums from nonsyphilitic persons and (5) serums from patients with diseases other than syphilis. All procedures in this test are carried out under strict aseptic precautions. All steps involving the exposure of the organism to adverse living conditions are carried out as quickly as possible to obtain optimal survival of the organism. The test procedure itself involves pipetting 1.7 cc. of filtered treponeme suspension (10^7) into a test tube of appropriate size and adding 0.2 cc. of serum to be tested and 0.1 cc. of guinea pig complement. Control tubes containing 1.7 cc. of treponeme suspension, 0.1 cc. of complement and 0.2 cc. of ultrafiltrate of serum are tested simultaneously. The tubes are incubated at 35 C. in Brewer anaerobic jars with 5 per

cent carbon dioxide and 95 per cent nitrogen. The percentage of motile organisms is determined by examining 50 successive treponemes as previously described. Counts were made at the start of the test and at four, eight, and sixteen hours. In the survey experiments counts were taken at sixteen hours, as this was the time showing optimal results. At the termination of each test failing to produce immobilization, the contents were tested for the presence of active complements in order to eliminate the possibility of false negative results.

Serums of healthy and of syphilitic rabbits had no immobilizing influence on T. pallidum in the absence of guinea pig complement. However, in the presence of complement there was a marked reduction in motility of the organism in the syphilitic serum pool but not in the normal serum. In normal serum with complement there was 96 per cent motility at twelve hours and 84 to 92 per cent motility at twenty-four hours in dilutions of 1 : 10 and 1 : 1,000 respectively. Syphilitic serum diluted with complement to a fluid dilution of 1 : 10 gave 2 per cent motility at twelve and twenty-four hours, while in a dilution of 1 : 1,000 there was motility of 92 and 84 per cent at twelve and twenty-four hours respectively. In control tubes there was 92 per cent motility for the same period. Since complement is necessary for immobilizing effect, this would suggest that immobilization is an antibody against T. pallidum.

Some previous tests showed that inactivated normal serum in concentration of 40 per cent produced a significant inhibition on survival of spirochetes in the absence of complement and some immobilization occurred in 20 per cent solution. However, in concentration of 1 :10 there were no immobilizing properties; thus this percentage and a minimum of sixteen hours' incubation was used in survey experiments. Therefore, in survey experiments all serums were employed in dilutions of 10 per cent, and mixtures were incubated at 35 C. for sixteen hours.

Treponeme suspensions with the designated amounts of serum and complement were subjected to temperatures of 25, 30, 35 and 40 C. for fifteen minutes. The rate of immobilization was determined by reading the percentage of motile organisms at four, eight and sixteen hours. Tests showed that the rate of antibody action increased with temperature; as a result the sensitivity of antibody action at any given time interval also became greater. Significant immobilization of the control was manifest as early as four hours at 40 C. but not at 35 C. or lower. Therefore, in subsequent survey experiments the incubation temperature of 35 C. was employed.

The immobilizing activity of syphilitic rabbits serum was tested with (*a*) untreated, (*b*) heat-inactivated and (*c*) decomplemented guinea pig serum. Two controls, one of saline solution and the other containing ultrafiltrate of serum, were set up. Tests showed that heat inactivation or removal of complement by absorption (decomplemented guinea pig

serum) with a specific precipitate renders guinea pig serum incapable of immobilizing T. pallidum in the presence of syphilitic rabbit serum. Therefore, the immobilizing activity of fresh guinea pig serum is attributed to the complement content. Immobilizing activity was manifest only in the presence of complement and was maximal when the final dilution of guinea pig serum was 1:10 to 1:20.

The authors found it necessary to prove that nonmotile organisms were dead in order to prove that the effect of the antibody was treponemicidal. Total counts of percentage of motile organisms in mixtures of treponemes, serum and complement were determined after incubation for sixteen hours at 30 C. Motility readings of complement with ultrafiltrate serum control was 80 per cent; of complement plus normal serum, 78 per cent, and of complement plus syphilitic serum, 4 per cent. Then 0.1 cc. portions of the suspensions were inoculated intracutaneously into the backs at four sites on each of 2 rabbits. This enabled the authors to compare the three samples in a single animal, eliminating the variation in individual response. Significant differences in the incubation period and the size of the resultant lesions were noted between the suspension of 4 per cent of motile organisms and the specimens containing 78 to 80 per cent of motile organisms. It was concluded that a significant proportion of the spirochetes in the mixture of syphilitic serum plus complement were rendered noninfectious or presumably dead, though the data do not give a quantitative estimate of the spirochetes so affected. It is also probable that the immobilizing antibody rendered the organisms more susceptible to the host's defense mechanism.

Using another batch of T. pallidum, a control tube of ultrafiltrate serum and complement with a 90 per cent motility after sixteen hours at 35 C. and a tube of syphilitic serum and complement with a 4 per cent motility rate, the authors did similar intracutaneous experiments. Lesions positive for treponemes on dark field examination developed in six days at the sites inoculated with the control suspension (90 per cent motile), whereas at only one site a similar lesion developed on the twenty-eighth day following the inoculation of the syphilitic serum–complement suspension (4 per cent motility). It was estimated that less than 500 viable treponemes were present in a total of 500,000, or 0.1 per cent, which would be compatible with 1 per cent motility in a count of 50 treponemes. This further indicated that immobilization by antibody and complement renders treponemes noninfectious or nonviable.

A series of 20 normal rabbit serums and 20 serums from untreated syphilitic rabbits were tested for immobilizing antibody and for reagin titer. Motility was determined after sixteen hours at 35 C. The controls showed 90 per cent motility; normal rabbit serums with complement had a motility range from 70 to 90 per cent, whereas syphilitic rabbit serums with complement had a motility range of 0 to 12 per cent. All

20 serums of syphilitic rabbits showed a marked degree of mobilizing activity. In the latter group there was no immobilization in the absence of complement.

Serums from 20 nonsyphilitic persons and from 20 patients with confirmed primary or secondary syphilis were tested, regardless of the serologic titer. In addition, serums were obtained from 20 patients suffering from diseases other than syphilis. Of the 20 patients with primary syphilis 7 had serums negative for immobilizing antibody; 10 had serums positive for such antibody and 3 had serums showing a slight degree of immobilization. All serums from patients with secondary syphilis showed a marked degree of immobilizing activity. Serums of nonsyphilitic persons showed no immobilizing activity. Of patients with disease other than syphilis, serums from 16 showed no immobilizing action, whereas serums from 4 did have immobilizing effect. These 4 patients had been receiving penicillin, and it is quite possible that they had sufficient of the antibiotic in the serum to inactivate the spirochetes. It is therefore advisable to obtain serums before antibiotics, such as bacitracin, penicillin and aureomycin, are administered.

In order to study the relation of reagin to immobilizing antibodies, absorption experiments were conducted. By means of two absorptions with use of Eagle antigen, lipid particles were removed from serologically positive serums by centrifugation and filtration, removing titratable reagin. In these serums subsequently tested for immobilizing antibody, there was no decrease in the immobilizing activity after removal of reagin. Repeated tests showed the same clearcut results, clearly indicating that immobilizing and reagin activities are due to separate antibodies.

Finally, it was demonstrated that reagin titer drops as the temperature increases but there is no corresponding decrease in immobilization activity. Whereas reagin titer drops progressively when syphilitic serum is exposed to temperatures of 56, 60, 64 and 68 C. respectively, the immobilizing antibody withstands temperatures as high as 70 and 72 C. The latter thermostability is unusual for antibodies, but such thermostability is noted in the flagellar component of hog cholera bacillus. An in vitro technic for the detection and measurement of specific antibody produced during a syphilitic infection of the immobilization antibody activity offers an approach to certain fundamental problems on syphilitic infection: (1) the incidence and titer of the immobilizing antibody in various stages of syphilitic infection; (2) the rate of appearance of the antibody following infection, and its quantitative relationship to the development of immunity as measured by resistance to infection; (3) the possible absence of immobilizing antibody in nonsyphilitic persons whose serums contain reagin or in the biologic false positive reactors; (4) the immunologic relationship in experimentally induced yaws and

other spirochetoses; (5) The possible immunologic variations among different strains of T. pallidum. It will be necessary to simplify and investigate thoroughly the technical aspects of the immobilizing antibody test before it can have practical applications. The chief value of this test would be in detection of the biologic false positive reactors.

Since this paper was published, and according to Nelson's presentation at the Syphilis Study Section in Washington, April 1949, his studies have been extended to include immobilizing activity of serums from patients with late syphilis, including neurosyphilis. These results indicate that the immobilizing antibody is just as clearcut as in the tests on serums of the present report.

PROGNOSIS OF SYPHILIS

In discussing the prognosis of syphilis, King [42] believes it is necessary to define what is meant by "cure" of syphilis. He states that "cure" in early syphilis means (1) clinical recovery which is maintained throughout life, (2) absence of infectivity to others and (3) persistently negative blood and spinal fluid reactions. In late syphilis "cure" is (1) permanent clinical recovery, (2) absence of infectivity, and (3) negative reactions in cerebrospinal fluid. Seroresistance of late syphilis is of no consequence in prognosis. However, should the cerebrospinal fluid reaction remain positive there is probability that neurosyphilis will develop and special treatment measures will be required. Early and late syphilis present different prognostic problems and are, therefore, considered separately.

In Great Britain adequate therapy is considered to be administration of penicillin and a full course of arsenical and bismuth preparations. Patients treated in this manner attain "cure," as previously defined, in 90 per cent or more of the cases. However, these patients should be followed at least for a two year period, and frequent serologic tests should be made. Thereafter examination and blood tests should be done annually throughout life.

Not only is inadequate treatment of early syphilis ineffective, but it interferes with the normal development of immunologic processes. In such a case serious late complications are more apt to develop. Consequently, inadequate treatment is probably worse than no treatment at all.

If cardiovascular and neurosyphilis do not develop, the course of late syphilis is benign, since apparently satisfactory immunologic processes develop. If lesions appear, they respond readily to antisyphilitic treatment. Late syphilis requires more treatment to insure against neurosyphilis or cardiovascular syphilis. Inadequately treated late syphilis per se apparently offers no harmful results. The prognosis of cardiovascular

42. King, A. J.: The Prognosis of Syphilis, in the British Encyclopaedia of Medical Practice, London, Butterworth & Co., Ltd., 1948, Interim Supp. **70**, pp. 12-13.

syphilis depends on the site and extent of the lesion. If aortitis is detected before vascular involvement the prognosis is good. Likewise the prognosis of neurosyphilis depends on site and severity of the lesion. Meningeal and vascular lesions have a better prognosis than parenchymatous syphilis. Treatment aims at arrest and symptomatic relief.

Simon [43] presents a compilation of old data on prognosis from Fournier, Audry, Jeanselme, Charcot, Fryer and others. No new viewpoints are advanced.

On the other hand, Blum and Barnett [44] made a comprehensive analysis of the prognosis for 2,566 patients with late syphilis in relation to treatment. They felt that the conventional methods of estimating progressions on a percentage basis and of requiring an arbitrary minimum period of observation before a patient is qualified for inclusion in the study led to erroneous conclusions. Although the original work should be consulted for detail, they proposed that progressions be expressed in relation to total years or centuries of patient observation, without a minimum period of observation, and the present analysis is presented in this manner. The prognosis in late latent syphilis appears to be improved by treatment at any time, and there seems to be steady improvement with increasing amounts of therapy. The conventional course treatment of twenty injections of an arsenical and twenty injections of a bismuth preparation is only about half of a truly adequate regimen. It is suggested that by the use of the proposed method of analysis it should be possible to determine the value of penicillin in latent syphilis in a few years if a large group of patients were treated, rather than in the ten to twenty-five years previously thought necessary for such a study.

Kahn and Iskrant,[45] statisticians of the United States Public Health Service, observed that the reported death rates from syphilis in the United States have been steadily reduced since passage of the National Venereal Disease Control Act in 1938. This trend is reliable since factors which would have an upward force on the rate, such as (1) improved reporting by physicians, (2) increased life expectancy and (3) redefinition of aneurysm as a syphilitic category, did not prevent this downward trend. This downward trend was noted in all types of syphilis among the white population and in all categories except paresis among the nonwhite. The mortality rate for sex and color groups show that (*a*) the rate for nonwhite persons is six times that for persons of

43. Simon, C.: Regards sur le domaine et le pronostic de la syphilis, Bull. Soc. franç. de dermat. et syph. **55**:140-143 (March-April) 1948.

44. Blum, H. L., and Barnett, C. W.: Prognosis in Late Latent Syphilis, Arch. Int. Med. **82**:393-409 (Oct.) 1948.

45. Kahn, H. A., and Iskrant, A. P.: Syphilis Mortality Analysis 1933-1945, J. Ven. Dis. Inform. **29**:193-200 (July) 1948.

the white race and (*b*) the rate for males is about three times that for females. These rates are quite different from those obtained by reported case rates—e. g., the rate for nonwhite was fourteen times that for white persons. The lost life years due to syphilis were 209,303 in 1933, as compared with 89,109 in 1944. There was decreased syphilis mortality in almost all the states, paralleling the national trend. The infant mortality rate for syphilis per 1,000 live births showed an over-all decrease, but the rate of decrease was greater than that of the general syphilis mortality rate. Color-specific syphilis mortality rates are much higher for the non-Southern states than for the Southern states.

The reader is referred for some of the more recent discussions of prognosis to the papers of Harrison,[46] Hahn and Clark [47] and Moore and Schamberg.[48]

SERODIAGNOSIS

Studies on the serology of syphilis have been rather numerous in the period covered by this review. It is twenty-five years since Kahn introduced the first practical precipitation procedure for the serologic diagnosis of syphilis. Activity, however, has been manifested chiefly in the evaluation of cardiolipin antigens, quantitative procedures and the nonspecific serologic reactions. Nelson and Mayer's fundamental study on immobilizing antibodies may, if properly applied, lead to the development of a procedure far superior to the present from the standpoint of specificity and sensitivity. A number of papers on the interpretation of the serologic reactions have been produced. Among these are the studies of Cope,[49] Frohbose [50] and Zwally.[51]

Certain tests are still promoted as screen procedures. For example, MacNab and Lewin,[52] in a long review of various laboratory procedures in the serodiagnosis of syphilis, make particular reference to the modified Ide screen test. The experimental details relating to the preparation of the antigen, use of the sensitizing reagents, use of gum benzoin, optimal

46. Harrison, L. W.: Venereal Diseases and Life Assurance, Brit. J. Ven. Dis. **16**:1-33 (Jan.-April) 1940.

47. Hahn, R. D., and Clark, E. G.: Asymptomatic Neurosyphilis: Prognosis, Am. J. Syph., Gonor. & Ven. Dis. **30**:513-548 (Nov.) 1946.

48. Moore, J. E., and Schamberg, I. L.: The Eligibility of Syphilitic Persons for Life Insurance, J. A. M. A. **134**:1532-1535 (Aug. 30) 1947.

49. Cope, H. E.: Interpretation of Serological Tests for Syphilis, J. Michigan M. Soc. **47**:1096-1099 (Oct.) 1948.

50. Frohbose, W. J.: Serologic Tests for Syphilis and Their Clinical Application, Virginia M. Monthly **75**:292-294 (June) 1948.

51. Zwally, M.: Recent Observations in the Serology of Syphilis, Am. J. M. Technol. **14**:224-227 (July) 1948.

52. MacNab, G. M., and Lewin, W.: Laboratory Procedures in the Serodiagnosis of Syphilis: An Improved Screen Test, South African M. J. **22**:677-683 (Nov. 13) 1948; 726-734 (Nov. 27) 1948.

proportions of antigen and serums, inactivation of serums, salt concentration and temperature in relation to the modified screen test are given in detail. Results obtained with 27,225 serums submitted to the screen test are recorded. Approximately 25 per cent of the serums yielded positive results to the screen test, and in no instance was an indisputable false negative result obtained.

In an editorial in the same journal [53] the fact is stressed that a laboratory test is a biochemical reaction occurring in a test tube and is not a diagnosis of a disease. The practitioner must consider laboratory results in conjunction with clinical findings before making a diagnosis of syphilis. With the large volume of specimens submitted for serologic tests, it has become the practice in major laboratories to submit all specimens to an initial screen test which is supersensitive in order to separate definite negative from all doubtful and positive reaction. The latter are then subjected to further confirmatory tests.

Sandiford and Khal [54] concluded from the study of 23,000 serums in the Laboratories Department, Ministry of Public Health, Cairo, Egypt, that the Laughlen test is reliable as a screen or parallel test. They found agreement in the Wassermann test and the Laughlen test in 92.5 per cent and disagreement in 7.5 per cent. More important, however, was the finding of a "false Laughlen negative" reaction (Laughlen reaction negative and Wassermann and Kahn reactions positive) in only 0.2 per cent of the total serums examined and in 1.1 per cent of the serums positive by both Wassermann and Kahn tests. They also found the Laughlen test suitable for examination of the cerebrospinal fluid. The degree of flocculation produced is not of much value as a quantitative result unless it is maximal or minimal.

Lane,[55] at the Shenley Hospital, Shenley, Herts, England, describes a rapid method for the diagnosis of syphilis by serologic reaction. His method employs the standard Meinicke (Ford Robertson–Colquhoun modification) antigen. Results are read after ten minutes of rocking in an incubator at 56 C., whereas results were read after eighteen hours in the original Meinicke test. One thousand specimens (858 of blood serum and 142 of cerebrospinal fluid) were subjected to the spot test in parallel with the Meinicke clarification test and the Wassermann test. The results with the rapid method (spot test) were in agreement with

53. The Screen Test for Syphilis, editorial, South African M. J. **22**:697-699 (Nov. 27) 1948.

54. Sandiford, B. R., and Khal, A. M.: The Laughlen Test with Particular Reference to Its Suitability as a Preliminary Screen Test, Brit. J. Ven. Dis. **24**: 72-75 (June) 1948.

55. Lane, R. F.: Spot Test for Syphilis Using the Modified Meinicke Antigen of Ford Robertson and Colquhoun, Arch. Dermat. & Syph. **57**:802-809 (May) 1948.

the Wassermann reaction in 97.5 per cent of the tests, whereas the results with the standard Meinicke test were in agreement with the Wassermann reaction in 98 per cent of the cases. Reactions to both the spot test and the routine Meinicke test tend to remain positive after the Wassermann reaction has become negative.

In an article which introduces a symposium in commemoration of the twenty-fifth anniversary of the Kahn test, Reuben L. Kahn [56] describes his early trials in a serologic laboratory. He noted four basic factors which govern the phenomenon of precipitation in syphilis, namely, (1) concentration of reagents, (2) antigen–salt solution suspension, (3) quantitative relation between serum and antigen suspensions and (4) agitation of the serum-antigen mixture. Kahn gives some concise comments on the serologic procedures developed in his laboratory for supplementary use to the standard Kahn test. The following are discussed briefly: (1) the presumptive test, (2) the quantitative test, (3) the qualitative spinal fluid test, (4) the quantitative spinal fluid test, (5) micro procedures for serum, spinal fluid, chancre fluid and vitreous humor, (6) optimal zone reactions in false negative cases, (7) quantitative optimal zone reactions, (8) verification reaction in false positive cases, (9) Kahn reactions with cardiolipin antigen and (10) the universal reaction with lipid antigen. All persons whose contact with serologic tests is only casual should read this article in the original so as to gain a clearer view of the numerous ramifications involved in serologic studies today.

The Expert Committee on Venereal Infections [57] has suggested that the World Health Organization might usefully seek to establish a uniform level of reactivity in serologic tests for syphilis as well as a repository for the resulting reference standard antigen. The committee recommended that a subcommittee on serology and laboratory aspects be established and that a preliminary program for the International Serological Laboratory Conference be drawn up. Since preliminary observations in certain countries have led to the belief that environmental factors may be responsible for a high percentage of positive serologic findings in some population groups in which the disease is comparatively rare, members of the expert committee were unanimous in recommending that the World Health Organization should undertake research on the relationship between positive serologic findings and seasonal and climatic factors, diet and standard of living and the simulataneous existence of other diseases.

56. Kahn, R. L.: Twenty-Five Years of Precipitation Studies in Syphilis (1923-1948), J. Michigan M. Soc. **47**:1089-1094 (Oct.) 1948.

57. Serology of Syphilis, Chronicle of the World Health Organization **3**:14-15 (Jan.) 1949.

In answer to varying opinions of several authors, Schröer [58] expresses the opinion that the complement fixation test for the diagnosis of syphilis can be replaced by the proper choice of a combination of flocculation tests. This opinion is based on results obtained with multiple serologic tests (seven) for syphilis on 17,000 serums. Schröer notes that in primary syphilis the more sensitive flocculation tests show positive reactions sooner than do the complement fixation and the less sensitive Sachs-Georgi flocculation test. He believes the complement fixation test does not differ qualitatively from the less sensitive Sachs-Georgi flocculation test. The combination of flocculation tests, to be used and suggested from this study are (1) the Sachs-Georgi test, which parallels the Wassermann test in sensitivity and specificity and gives fewer nonspecific reactions; (2) the Meinicke clearing reaction, a more sensitive test, giving a higher percentage of nonspecific reactions; (3) the sensitive Kahn reaction. A combination of flocculation tests is suggested for more accurate serodiagnosis, detection and assurance of treatment in the positive cases. The author states that there is no practical reason for maintaining the complement fixation test.

Carter [59] details the responsibilities of both the serologist and the physician in holding serologic conflict to a minimum. She divides the reasons for serologic discord into three categories: (1) those inherent in the technical procedures; (2) those which are factors of the serum in relation to reagin content or independent of it, and (3) those which involve the condition of the person whose blood is being tested.

Cannefax [60] presents a method of preservation of serum for a positive control in the flocculation complement fixation tests, colloidal gold reactions and spinal fluid protein determinations. He points out the necessity of a standard control serum to control the sensitivity of quantitative and qualitative tests. Employing the method of preservation proposed, serum was maintained satisfactorily at room temperature for one year. Serum with a reagin titer of 512 Kahn units was precipitated and the lipids removed by the Hardy-Gardiner method at — 20 C. by a mixture of dehydrated alcohol (absolute ethanol) and absolute ether (diethyl ether) after the serum was concentrated to one third of its volume by passage through a cellophane tubing as described by Kober. The precipitated serum was filtered and washed several times with cold absolute ether. The ether and precipitate were removed in a vacuum

58. Schröer, W.: Kann bei der serologischen Luesdiagnose im Blutserum die Komplimentbindungsreaktion nach Wassermann ersetzt werden? Klin. Med. **3**: 623-631 (Aug. 15) 1948.

59. Carter, B. B.: Serologic Conflict, A Study of Serodiagnostic Discrepancies, Arch. Dermat. & Syph. **57**:368-373 (March) 1948.

60. Cannefax, G. R.: A Stable Control Serum for Standardizing the Sensitivity of Tests Used in the Diagnosis and Control of Syphilis, J. Ven. Dis. Inform. **30**:169-174 (June) 1949.

desiccator over sulfuric acid. The dry precipitate was reconstituted to the original volume of serum, divided into 5 cc. quantities and placed in ampuls and shell frozen with ethyl alcohol and solid carbon dioxide, followed by dehydration with the cryochem apparatus. Prior to sealing all residual moisture was removed by a vacuum apparatus. The processed serum was used daily for over a year for comparing sensitivities of Kahn quantitative, Kolmer quantitative and spinal fluid tests, quantitative and qualitative Venereal Disease Research Laboratory (VDRL) slide tests and colloidal mastic tests and as a control for turbidimetric quantitative determination of spinal fluid protein. Testing the control serum with 2,000 mixes of Kahn antigen and 236 VDRL antigen suspensions showed that the control serum possessed stability that was satisfactory. In the Kolmer tests it was necessary to shorten or lengthen the secondary incubation period, depending on the sensitivity of the hemolytic system, but the results were satisfactory. Colloidal mastic tests showed that the control serum maintained satisfactory stability after preservation for a year. Micro-Kjeldahl analysis of total protein in the control serum after one year added further evidence of the stability of the serum.

Kahn and McDermott [61] present a summary of the specificity results of the standard Kahn tests in all of the official evaluation studies in which the tests were performed by Kahn or in his laboratory. In the eighteenth evaluation studies (1928 to 1948) in which 3,740 nonsyphilitic donors were examined, the Kahn test gave one positive reaction and twelve doubtful reactions. The positive reactor similarly had positive reactions with all the other methods which were used in that study, and the belief is that the reaction was most likely specific. A table shows that since 1937 the Kahn test has not given any false positive or false doubtful reactions in Kahn's studies. Kahn and McDermott do not claim that the Kahn test is totally free from the possibility of giving false positive results, but no other test, neither complement fixation nor precipitation, has given such a high record of specificity in the evaluation studies. The question always arises in connection with Kahn and McDermott's claims whether the low degree of nonspecificity may be due to the low level of sensitivity which Kahn has maintained for his test over the years.

Boersma and Williams,[62] employing the "optimal zone procedure" described by Kahn, studied 26 patients with neurosyphilis who gave negative serologic reactions. Twenty-one of these patients gave positive

61. Kahn, R. L., and McDermott, E. B.: Specificity of the Kahn Reaction, J. Michigan M. Soc. **47**:1095 (Oct.) 1948.

62. Boersma, D., and Williams, P. G.: The Kahn Optimal Zone Reaction in Seronegative Cases of Neurosyphilis, Am. J. Syph., Gonor. & Ven. Dis. **32**:370-376 (July) 1948.

"optimal zone reactions." These reactions were limited to zone III, in which the serum is in excess of the antigen, the serum being relatively low in reagin content. Serums from nonsyphilitic persons used as controls gave negative "optimal zone reactions." The authors believe that a negative reaction to a serodiagnostic test may not mean total absence of serum reagin, and small quantities of reagin may be detected by the "optimal zone reaction."

Inhibitor of Serologic Reactions.—Volkin [63] reviews briefly certain human serum protein fractions which inhibit biologic false positive reactions in tests for syphilis. He describes also the removal of the inhibitory factor from certain serum fractions by alcohol extraction, the isolation of the inhibitor from the alcohol extract and its probable identity with human serum lecithin. Several samples obtained from the lipid fractionation of fraction IV-1 were tested for inhibitory activity against the euglobulin fractions of a biologic false positive and a syphilitic serum. It was demonstrated that the factor which inhibited the biologic false positive serologic reactions was contained in the acetone-insoluble, the cadmium chloride–insoluble, and the barium chloride–soluble fractions. The conclusion was drawn that this factor is a human serum lecithin because of its solubility behavior in acetone, cadmium chloride and barium chloride and its nitrogen: phosphorus ratio of unity. This human serum lecithin exhibited inhibitory activity toward euglobulin fractions of biologic false positive origin in lower concentrations than the whole serum fraction IV-1. Comparative measurements, however, indicated that the inhibitory activity of the latter was more specific. The author believes that the higher specificity of the whole fraction IV-1 is due to the firm combination of the serum lecithin with the protein. Human serum lecithin and purified beef heart lecithin have approximately the same specific inhibitory activity; egg lecithin, however, has no specific inhibitory activity.

Complement.—Ecker [64] presents a broad appraisal of the newer studies as well as a survey of the investigations and activity of complement. Available evidence indicates that, not unlike guinea pig complement, human complement is composed of four components, the main difference being that, whereas in the guinea pig fraction C′ 3 is associated with fraction C′ 1, and C′ 4 with C′ 2, in the human being both C′ 3 and C′ 4 are in persistent association with C′ 1 and C′ 2. Human complement was characterized by Pillemer as a euglobulin with an electrophoretic mobility of 2.9×10^{-5} in veronal buffer. Examined in the Svedberg oil turbine ultracentrifuge, the C′ 1 fraction was found

63. Volkin, E.: The Isolation of a Serum Lipid Which Inhibits Serologic Reactions, J. Immunol. **61**:143-151 (Feb.) 1949.

64. Ecker, E. E.: Complement, Ann. Rev. Microbiol. **2**:255-268, 1948.

to contain a major component which had a sedimentation constant of 6.9 Svedberg units. It constitutes about 0.6 to 0.8 per cent of the total serum protein. Cohn and co-workers have obtained the human C′ component in their fraction III-2. The C′ component is inactive per se in both hemolytic and bacterial systems but will react in combination with the three other fractions. Human C′ 1 fraction was further subjected to chemical analysis by Ecker and associates. This fraction contained 4.26 per cent polysaccharides and 23.4 per cent hydroxyamino acids. The C′ 2 fraction was also obtained in a highly purified form by precipitation of human serum at p_H 6.8 with 2.4 to 2.6 molar ammonium sulfate solution. It was found in this laboratory that, although it has been difficult to remove all the albumin from this fraction, a globulin was obtained having an electrophoretic mobility of 2.6×10^{-5} in a veronal buffer of p_H 7.8 and an ionic strength of 0.1. This globulin contained about 2 per cent carbohydrate. By itself C′ 2 was inactive, but it was fully active when in combination with the other three components. Except for the fact that C′ 3 is one of the minimum and relatively labile components of the complex, little is known about this fraction. Human C′ 4 was also prepared relatively free from the other components; it is highly sensitive to dilute bases and amino compounds. Doxois, Seifter and Ecker showed that the killing of Vibrio comma by fresh human serum is caused by the combined action of antibody and complement and that destruction of any one of the four components leads to a loss of bactericidal effect. Evidence was also obtained pointing to the identity of the bactericidal and hemolytic complements. Doxois, Seifter and Ecker found that in bacterial complement fixation inactivation occurs by virtue of the removal of one or more of the components. C′ 2 and C′ 3 were the components principally bound.

Bergenhem observed that at 39 C. human complement lost practically all its activity in a period of ten hours and that in "heat stabilization" C′ 3 was the component destroyed. According to Gordon and Walker, "inactivation of guinea pig complement by dyestuffs (congo red series) is not associated with any important chemical changes in the constitution of the compounds. Adsorption or loose union occurs and the effect appears to be dependent more on the molecular size than on any specific grouping. Fibers of cotton and silk reverse the action." Boursnell and associates reported that small amounts of mustard gas rapidly inactivate complement, affecting all the components, and especially C′ 1 and C′ 2. Divinylsulfone inactivates complement even more rapidly. β′β′-dichlorodiethylsulfone operates more slowly. Further work is needed to discover whether the effect in this case is due to an oxidation of the SH groups by the β′β′-dichlorodiethylsulfone. The coupling of proteins with sulfones mentioned produces derivatives with altered immunologic specificity. Mayer reported evidence indicating that the magnesium, calcium, nickel or cobalt ion is essential for the hemolytic action of com-

plement and that the enhancing action of tissue fluids can also be ascribed to the magnesium ion. In fixation tests for syphilis, however, the enhancing effect of the magnesium ion was neutralized by the inactivated human test serum. Failure to take account of this effect for the magnesium ion treated complement may lead to errors in the evaluation of titer. According to Heidelberger and Mayer the function of complement does not appear to be enzymatic in nature. The kinetics of the action of complement on sensitized erythrocytes has been studied by Meyer and Craft, who obtained evidence showing that the antibody resembles an enzyme, whereas complement operates as a co-factor which is used up in the process. Jordan claims that there is no direct connection between the complementary power of a given serum and its anticomplementary action. Highly anticomplementary gamma globulins were separated electrophoretically from normal human serums by Davis and associates. Purified syphilitic antibody, prepared by dissociation of the specific precipitate, proved to be anticomplementary in a similar fashion. Anticomplementary action was decreased by heating at 56 C. for thirty minutes, and abolished by the addition of approximately equal amounts of albumin or beta globulin. It is known, however, that some serums develop the anticomplementary power only after heating. By electrophoresis and by chemical fractionation it was possible to isolate the relatively thermostabile anticomplementary globulin. The separated fraction appeared to be a pseudoglobulin with a molecular weight of 170,000 which is considered higher than that of the normal gamma globulin. Olhagen believes "the anticomplementary effect is due to a form of denaturation of the globulin involving a change in dispersion which leads to complement adsorption. Of 404 cases of liver disease and allied conditions 18.8 per cent exhibited anticomplementary effects. One third of these sera originated from cases of cirrhosis and chronic or relapsing hepatitis." On the basis of this study, Olhagen recommends that the occurrence of anticomplementary power in a heated serum be given clinical consideration. Erickson found that the reactive bodies in both syphilitic serums and in serums giving biologic false positive reactions are exclusively associated with the gamma globulin, the faster moving "half" of the gamma globulin being serologically more active per milligram of protein than either the whole or the slow moving "half".

Ecker, Hiatt and Barr observed that in pregnant guinea pigs substantially lower complement activities occur than in nonpregnant animals and that in the postpartum period there is a gradual rise in complement activity approaching a level normal for nonpregnant females. A marked reduction in complement activity was observed by Dulaney in 24 patients with neurosyphilis treated with malaria. No marked variation from the normal was noted in the serums of 25 patients with hepatic disease or in 32 patients with noninfectious diseases of various types. Of 9 dogs with

alloxan diabetes, Candela and Urgoiti found that 7 showed a diminution of complement varying between 12 and 68 per cent. Age, sex and season of the year have no effect on complement activity.

Sheep Cells.—Muraschi and Tompkins [65] used pooled preserved sheep blood (pretested for compatibility) as a source of erythrocytes in the quantitative complement fixation test. They report that they observed a uniform activity not obtained with blood from a single sheep and that the standardization of the hemolytic system has been greatly simplified. They used merthiolate® in concentrations 1:2,000 and 1:10,000 for maintenance of sterility of the citrate-glucose solution in which the cells were collected. This added chemical did not increase the tendency toward hemolysis on washing or in titration with complement and amboceptor.

Cardiolipin Antigen.—With little exception, chiefly on the part of Kahn, cardiolipin antigens have been widely applied with apparently favorable results. We review some of the articles on the year's efforts.

After a cardiolipin-lecithin antigen was found to be satisfactory in complement fixation and flocculation tests for syphilis, a microflocculation test, or the VDRL slide flocculation test, was described. Now Harris, Rosenberg and Del Vecchio [66] describe in detail a tube quantitative and qualitative macroflocculation test using the cardiolipin-lecithin antigen. This test is referred to as the VDRL tube flocculation test. Comparison of results obtained with the VDRL slide and tube flocculation tests on a series of 5,016 serums shows that the tube test operates at a reactivity level approximating that of the VDRL slide flocculation test. This tube test is offered as a companion test to the slide test and not as a substitute.

Levin [67] discusses the preparation of the Kahn, Kline and Kolmer cardiolipin antigen. Each serologist has his own opinion as to the efficiency of the respective antigens, and each has a personal method of preparation of the antigen and technic of performing the serologic test. Levin is of the opinion that cardiolipin antigen compares favorably with other antigens in both sensitivity and specificity. It is the consensus that the antigen is more specific but less sensitive. Kahn reports that the sensitivity of the cardiolipin antigen approaches that of the Kahn antigen especially if centrifugation is applied to borderline reactions. Levin's experience shows that the Kahn cardiolipin antigen has a

65. Muraschi, T. F., and Tompkins, V. N.: Pooled Sheep Cells for Use in Hemolytic System of the Complement-Fixation Test, Am. J. Clin. Path. **19**:152-155 (Feb.) 1949.

66. Harris, A.; Rosenberg, A. A., and Del Vecchio, E. R.: A Macroflocculation Test for Syphilis Using Cardiolipin-Lecithin Antigen, J. Ven. Dis. Inform. **29**:313-316 (Oct.) 1948.

67. Levin, W.: Present Status of Cardiolipin Antigen in Syphilis Serology, Northwest. Med. **47**:659-662 (Sept.) 1948.

sensitivity definitely below that of the standard Kahn antigen and is inferior to the Kline cardiolipin and the VDRL slide test, while its sensitivity is greater than that of the Kahn macroflocculation test. The cardiolipins give false positive reactions, as do other antigens. Kolmer has found that his cardiolipin antigen gives false positive and doubtful reactions in leprosy, viral pneumonia, infections of the respiratory tract, vaccinia and infectious mononucleosis, but it gives a lower proportion of false positive reactions in malaria. Kline stated that the Kline cardiolipin in slide tests gave more specific reactions in nonsyphilitic conditions than did Eagle, Hinton, Kahn, Kline and Mazzini antigens. In a series of 15,000 comparative tests in 1946-1947 the VDRL cardiolipin slide test agreed 97.6 per cent with the Kahn test and 98.4 per cent with the Kline test. Levin states that there is much to be done in the way of refinement and possibly technic before any of the proved procedures can be discarded for a single cardiolipin antigen method.

The VDRL slide test has been compared by Dammin and Scott[68] with the standard Kahn test in a study of 1,800 serums. The cardiolipin test often elicited positive reactions in slightly lower dilutions than did the quantitative Kahn test. However, of 9 patients with Kahn titers of 40 units or higher and negative reactions with cardiolipin, 7 were found not to have syphilis, 1 was suspected of having syphilis and 1 had late latent syphilis. There were 4 in whom the cardiolipin reaction was positive and the Kahn reaction negative. All were found to have been treated for late syphilis. The data accumulated during this experience would indicate the VDRL cardiolipin slide test to be simpler, more sensitive and more specific than the standard Kahn test.

Vogelsang[69] reports the results of serologic tests—the Bordet-Wassermann reaction carried out with the cardiolipin-lecithin-cholesterol antigen. The test performed with Pangborn's preparation of antigen containing cardiolipin 0.0175 per cent, lecithin 0.0875 per cent and cholesterol 0.3 per cent was found to be as sensitive as that carried out with the cholesterolized extract of ox heart. The Bordet-Wassermann test carried out with cardiolipin-lecithin-cholesterol antigen gave fewer false positive reactions than did that carried out with ordinary cholesterolized extract of ox heart. Among 700 serums from patients with syphilis they found that the reactions of 401 were positive to cardiolipin antigen; 367, positive to extract of ox heart; 378, positive to Kahn antigen, and 432

68. Dammin, G. J., and Scott, V. C.: Experiences With Cardiolipin Antigen, J. Missouri M. A. **45**:900-902 (Dec.) 1948.

69. Vogelsang, T. M.: Bordet-Wassermann's Reaction Carried Out with Cardiolipin-Lecithin-Cholesterol Antigen: A Preliminary Report, Acta path. et microbiol. Scandinav. **25**:167-174, 1948.

positive to Meinicke antigen. Among 4,930 serums from nonsyphilitic patients, the reactions of 36 were positive to cardiolipin antigen; 59 were positive to extract of ox heart; 51 were positive to Kahn antigen, and 67 were positive to Meinicke antigen. Further, cardiolipin-lecithin-cholesterol antigen possesses one advantage in that it can be easily standardized, and its general use in different laboratories should, therefore, help to give more uniform results.

Klein and Leiby [70] tested 3,800 serum samples submitted for routine testing for syphilis. The cardiolipin slide test employing a cardiolipin-lecithin ratio of 1:10 as described by Kline had a specificity of 97 per cent, as compared with a specificity of 98.4 per cent with the standard Kahn test. The sensitivity of the cardiolipin-lecithin in the ratio of 1:10 was 99 per cent. A cardiolipin-lecithin ratio of 1:8 gave maximal sensitivity and specificity, closely approaching that of the Kahn test. This test had striking specificity in a group of patients with malaria. Later Klein, Leiby and Berke [71] found it desirable to modify Kline's recommended cardiolipin-lecithin ratio in order to get results of optimal specificity with the Kline cardiolipin slide test. They recommend a cardiolipin-lecithin ratio of 1:8. With this they showed in 4,718 tests an improved and practicable level of specificity, only slightly less than with the standard Kahn test, and significantly higher sensitivity results than did either the Kahn or the Kolmer tests.

In previous reports on the adjustment of cardiolipin-lecithin-cholesterol antigen, Brown [72] reported that maximum reactivity occurred when the ratio of lecithin to cardiolipin was between 10:1 and 25:1. When antigens of varying proportions within this range were tested with dilutions of a standard pool of reacting serums, the maximum reactivity seemed to depend on the total concentration of lipid rather than on the proportion of lecithin to cardiolipin. With undiluted serums, however, as the proportion of lecithin to cardiolipin increased from 10:1 to 25:1, the tendency of high titer serums to give prozone reactions and inhibition phenomenon decreased. The experimental antigen in the present series of tests had a ratio of 10 parts lecithin (0.75 per cent) to 1 part cardiolipin (0.075 per cent), whereas the routine antigen was 25 (0.75 per cent) :1 (0.03 per cent) respectively. Cholesterol was present in the concentration of 0.25 per cent in each. The experimental

70. Klein, S. J., and Leiby, G. M.: Cardiolipin Slide Test in the Serodiagnosis of Syphilis, Am. J. Syph., Gonor. & Ven. Dis. **32**:377-390 (July) 1948.

71. Klein, S. J.; Leiby, G. M., and Berke, M.: Cardiolipin Antigen in the Kline Test for Syphilis, Am. J. Clin. Path. **18**:940-952 (Dec.) 1948.

72. Brown, R.: Cardiolipin-Lecithin-Cholesterol Antigen in the Precipitation Test for Syphilis; Influence of Ratio of Lecithin to Cardiolipin on Antigen Activity, Am. J. Clin. Path. **18**:565-567 (July) 1948.

antigen failed to give quantitative results in tests employing diluted serums. The precipitates formed in tests using the experimental antigen were unstabile and tended to disappear on shaking. The excess of cardiolipin seems to have a dispersing effect on the precipitate in the test. Furthermore, specimens of nonreacting serums appear much less granular than those in routine tests, which may be the effect of the marked peptizing action which cardiolipin in small amounts exerts on cholesterol crystals. The experimental antigen occasionally reacts to a slight degree with serum from a case of syphilis at an earlier stage than does the routine antigen.

McDearman and Cottrell [73] performed a total of 60,226 serologic tests on 15,019 serums. They obtained weakly positive or positive cardiolipin reactions and negative Kahn and Kolmer reactions with 991 serums representing 654 persons. Of 454 patients for whom adequate clinical and social histories were available, 43.2 per cent gave an acceptable history of previous syphilitic infection or clinical evidence of a current lesion. They believe it is probable that an additional number of persons reacting to cardiolipin were, or previously had been, syphilitic. They base the latter statement on a clinical history suggestive of syphilis, a history of other venereal disease, a low incidence of diseases commonly known to give false positive reactions for syphilis and the similarity of the pattern of reaction in the "negative" group to that in the syphilitic group.

Kolmer and Lynch [74] report improvement in the Kolmer antigen for use in the complement fixation test by changing the technic of its preparation. With this improved antigen it was not necessary to pretest complement; prezone reactions with human serums have been practically eliminated, and it was found unnecessary to use egg albumin for the prevention of falsely positive reactions with normal spinal fluids. After testing thirty-nine different combinations of cardiolipin, lecithin and cholesterol as antigens in the Kolmer complement fixation test, Kolmer and Lynch preferred an antigen prepared from 0.03 per cent cardiolipin, 0.05 per cent lecithin and 0.6 per cent cholesterol in dose of 0.5 cc. of 1:150 dilutions. Both antigens give practically 100 per cent negative reactions in the Kolmer complement fixation test employing the serums and spinal fluids of presumably normal nonsyphilitic persons. Cardiolipin and improved Kolmer antigens are closely similar in antigenic sensitivity, although the former may give from 1.0 to 1.5 per cent more positive reactions with the serums of syphilitic donors. Cardiolipin

73. McDearman, S. C., and Cottrell, J. E.: Specificity of Cardiolipin Antigens in Tests for Syphilis, Am. J. Clin. Path. **19**:156-162 (Feb.) 1949.

74. Kolmer, J. A., and Lynch, E. R.: Cardiolipin Kolmer Antigens in the Complement Fixation Test for Syphilis, Texas State J. Med. **44**:312-316 (Aug.) 1948; An Improved Antigen for the Kolmer Complement-Fixation Test for Syphilis, Am. J. Clin. Path. **18**:731-736 (Sept.) 1948.

antigen gives a lower incidence of falsely positive and doubtful reactions in malaria than does the improved Kolmer antigen and may be preferred in those localities where malaria is endemic. Both antigens give biologic falsely positive and doubtful reactions with serums from patients with leprosy, virus pneumonia, infections of the upper respiratory tract or vaccinia, as well as with normal rabbit serum.

A comparison was made by Giordano and his co-workers [75] between cardiolipin antigen and Kolmer antigen in the complement fixation test for syphilis. The study comprises two or more complement fixation tests on each of 1,295 serums examined during a ten month period and an evaluation of the results in terms of the clinical histories of the patients. Most of these specimens, of which 45.2 per cent were from syphilitic patients, were selected after screening by the microflocculation test. The complement fixation technic used was essentially that of Kolmer. The cardiolipin antigen consisted of cardiolipin 0.06 per cent, lecithin 0.05 per cent and cholesterol 0.9 per cent. Two saline solutions (one with distilled water and one with tap water) were prepared daily, and proportional amounts of the solutions were mixed to yield a solution of p_H 7.4 (methyl red indicator). The VDRL micro flocculation test was used routinely for screening and for comparison with the complement-fixation test using cardiolipin antigen. From this study it was concluded that the optimum titer of complement fixation antigen should be determined by routine clinical testing after block antigen titration with pools of diluted serums. Complement fixation tests with cardiolipin antigens show increased sensitivity with only negligible loss of specificity when compared with complement fixation tests using standard Kolmer antigen. The sensitivity of complement fixation tests using cardiolipin antigen approaches that of flocculation tests using cardiolipin antigen, without appreciable loss of specificity. In the interpretation of these data the authors emphasize the fact that a large proportion of the total number of serums was from patients known to have syphilis and from others for whom the diagnosis was undetermined. During the last three and one-half months of the study comparative studies also were made on Kolmer's new cholesterol-lecithin antigen, the results of which suggest that complement fixation antigens can be made more sensitive by the addition of cholesterol and lecithin. The new Kolmer cholesterol-lecithin antigen compared favorably with the cardiolipin antigen in the complement-fixation test for syphilis. The authors note that the new improved Kolmer antigen can be made in a well equipped serologic laboratory, but the preparation of cardiolipin and lecithin solutions require special facilities not often available.

75. Giordano, A. S.; Frost, R. J., and Higginbotham, M. W.: Cardiolipin Antigen in the Kolmer Complement-Fixation Test: A Comparison with Kolmer Antigen, Am. J. Clin. Path. **19**:25-29 (Jan.) 1949.

Observations by Kahn and McDermott [76] showed that different lots of cardiolipin antigen prepared by the same formula gave inconstant results in the Kahn standard test. The variables were present in either cardiolipin or lecithin or in both. Thus these were investigated in a series of experiments using different lots of lecithin with the same lot of cardiolipin and vice versa. Results showed that different lots of purified lecithin in cardiolipin antigen may cause the antigen to vary in sensitivity up to 40 per cent or more. These differences are best noted when weakly positive serums are employed. Preliminary standardization for cardiolipin antigen indicates that many purified lecithins will become usable by variations in the ratio of lecithin to cardiolipin within certain limits. Thus with use of a 10: 1 ratio of lecithin to cardiolipin as a working base, a reduction in lecithin to 9: 1 tends to decrease sensitivity, whereas an increase in lecithin to 11: 1 tends to increase sensitivity. Therefore, cardiolipin antigens prepared with different lots of purified lecithin require serologic standardization before their employment in tests for syphilis. Methods of standardization employed by Kahn and McDermott are detailed.

Quantitative Serologic Tests.—The quantitation of serologic results has been widely applied to the therapy of syphilis by foreshortened methods, especially use of penicillin. Both precipitation and complement fixation procedures have been modified to yield quantitative results.

Vogt [77] presents a method for the titration of serums which have shown 4 plus reactions on the Meinicke clarification test. The purpose was to establish a difference between true and false (or so-called overstrong) 4 plus reactions. One hundred serums were titrated in a geometric series in which every dilution was one fourth of the former. Sixty serums appeared negative and 40 positive after the titration, in agreement with the Bordet-Wassermann reaction and the Wadsworth and Brown reaction, which tests were performed simultaneously on the same serums.

Osler and Widelock [78] describe a rapid slide flocculation test for the titration of antibodies in syphilitic serums. This technic facilitates estimation of antibody titers by varying the amount of both serum and antigen, thereby eliminating the tedious and the time-consuming tube dilution procedure. To evaluate this new procedure, comparative titra-

76. Kahn, R. L., and McDermott, E. B.: Studies on Cardiolipin Antigen: Variations in Sensitivity of Different Lots of Purified Lecithin, J. Lab. & Clin. Med. **83**:1220-1223 (Oct.) 1948. McDermott, E. B., and Kahn, R. L.: Studies on Cardiolipin Antigen; Standardization of Antigen for the Kahn Test, ibid. **83**: 1224-1231 (Oct.) 1948.

77. Vogt, E.: The Meinicke Clarification Test, Acta dermat.-venereol. **29**: 259-265, 1949.

78. Osler, A. G., and Widelock, D.: A Rapid Slide Method for the Titration of Antibodies in Syphilitic Serum, J. Ven. Dis. Inform. **29**:239-242 (Aug.) 1948.

tions were done on a total of 400 syphilitic serums by employing this new rapid slide technic and tube dilution method. Identical titers were obtained in 73 per cent of the serums tested. Only 5 of the 400 serums showed a difference of titer greater than 1 dilution. In 25 per cent, or 102 specimens, there was a difference in titer equivalent to 1 dilution. Of these 102 specimens, 87 showed a higher titer of 1 dilution. This rapid slide test requires only 0.25 cc. of serum, and is, therefore, of practical importance so far as specimens from infants are concerned. The antigen used is the cardiolipin-lecithin-cholesterol mixture as prepared for the Venereal Disease Research Laboratory, United States Marine Hospital, Staten Island, N. Y. Substitution of the cardiolipin antigen by the Mazzini antigen yielded essentially the same results. This test may be satisfactorily substituted for the time-consuming tube dilution technic. Practically speaking, (1) this test requires only 0.25 cc. of serum, and (2) it is rapid and results compare favorably with those of the tests using tube dilution technic and should, therefore, be of value in laboratories doing mass titrations.

Kile [79] believes that quantitative tests for syphilis are advantageous when a prezone phenomenon is exhibited as well as in the diagnosis of prenatal or congenital syphilis.

Reporting from Western Reserve University, Ecker and Hiatt [80] present a highly technical paper describing a method for the quantitative measurement of complement fixation. Their method is exemplified in the quantitative Wassermann test described.

Prozone Phenomenon.—Beelar, Zimmerman and Manchester [81] report 5 cases of late syphilis with the prozone phenomenon. On the basis of these cases the authors state that two diagnostic tests of varying sensitivity should be employed routinely in the serodiagnosis of syphilis. If this is impractical or if clinical manifestations suggest the possibility of a syphilitic process despite negative or equivocal results, quantitative serologic tests should be done.

Biologic False Positive Reactions.—The various conditions in which the biologic false positive serologic tests for syphilis are manifest are discussed by Neurath.[82] The criteria for a biologic false positive reaction are set forth. The author then discusses cardiolipin and the euglobulin

79. Kile, R. L.: Quantitative Serological Tests in Syphilis, Christ Hosp. M. Bull. **2**:8-10 (July) 1948.

80. Ecker, E. E., and Hiatt, C. W.: A Method for the Quantitative Measurement of Complement-Fixation, Am. J. Clin. Path. **19**:141-151 (Feb.) 1949.

81. Beelar, V. P.; Zimmerman, H. J., and Manchester, B.: Prozone Phenomenon in the Serodiagnosis of Syphilis, Am. J. M. Sc. **217**:658-665 (June) 1949.

82. Neurath, H.: Biologic False Positive Reactions in Serologic Tests for Syphilis, Am. J. Med. **5**:670-678 (Nov.) 1948.

inhibition test (described in the 1948 review [1]). These two recent developments in the serologic field are promising tools to aid the clinician in the diagnosis of syphilis and curtail the incidence of biologic false positive reactions. These tests, however, have not reached the stage that they can replace the clinical examination and probably never will reach it. The introduction of cardiolipin in serologic tests for syphilis represents progress in the attainment of two goals: the standardization and reproducibility of antigen and an increased sensitivity and specificity for serologic tests. However, it has not shed light on the biologic false positive reactions, though the number has been lessened. The euglobulin inhibition test has been shown to distinguish biologic false positive reactions in more than 90 per cent of serums from carefully selected persons who have never had syphilis and to detect syphilis in 95 per cent of all serums from patients who have been known to have the disease.

Stokes and James [83] found that of 68 patients for whom a diagnosis of nonspecific positive reaction had been considered 23 were eliminated because an ultimate diagnosis of syphilis was made. They feel that the more critically it is examined the more complicated and puzzling the biologic false or nonspecific positive serologic reaction for syphilis becomes. It appears to be part of a general infection reaction mechanism shared by many diseases and forms of injury and more developed in some persons than in others (biologic false positive reactors). Febrile states, especially those due to infections; virus diseases (of the naso-respiratory and gastrointestinal tracts especially); foreign protein (disease-preventive) inoculations, and certain diseases such as leprosy, malaria and infectious mononucleosis are particularly prone to bring out biologic false positive reactions and reactors. There is reason to suspect that a seronegative status in a syphilitic person may be converted into a seropositive state by some of the agencies that can produce a biologic false positive state, and that the reactions of a patient with early syphilis approaching serologic cure may be maintained for some time in a low titer fluctuation, possibly by a biologic false positive reactor tendency. The proportion of biologic false or nonspecific positive reactions in a series of routine tests may approach 40 per cent of the positive reactions obtained in a run or survey. But the proportion of biologic false positive reactions in all tests, positive and negative, in serologic surveys probably does not exceed 1 in 700 tests. It is apparent, therefore, that there can be no excuse for discarding or minimizing the serologic tests for syphilis because there are biologic or nonspecific positive reactions. An effort should be made to repeat every test with a positive reaction; to check the commoner possibilities of biologic false positive

83. Stokes, J. H., and James, G. W.: The Problem of the "Biologic False" or Nonspecific Positive Serologic Test for Syphilis: Clinical Observations and Case Studies, Am. J. Syph., Gonor. & Ven. Dis. **33**:114-125 (March) 1949.

reaction at the time as well as after the test result is reported, and to give no treatment until a genuine attempt to evaluate the situation has been made. The major exception might be in the case of a pregnant woman approaching term. It cannot be denied that the evaluation of nonspecific positive reactions may be a prolonged, elaborate and expensive process and that the final result with present methods and knowledge may be inconclusive. Nonetheless, the effort should be made. With the help of multiple tests, the cardiolipin antigen and special procedures like the Neurath euglobulin-inhibition test, there is reasonable ground for hoping that an improved short and dependable differential procedure may be developed. The temptation to use 10,000,000 units of penicillin as a short-cut to a decision (accepting syphilis as the diagnosis) or to give it "just in case," is becoming more general and should be resisted as long as possible. The decision to fasten a diagnosis of syphilis on a patient who does not have it should not be lightly made. The decision to treat must be reached only after every possibility has been investigated and the serologic pattern studied over as long a time and by as many means as possible.

Moon-Adams [84] reports on 470 infants of treated syphilitic mothers examined within the first three months of life for serologic, clinical and roentgenologic evidence of syphilis. False positive serologic reactions were found in 69 (47.3 per cent) of 146 infants tested in the first month of life; in 16 per cent of infants tested in the second month of life, and in 1 of 87 (1.2 per cent) infants tested in the third month of life. False negative reactions were found in 5 infants, or 1 per cent of the total (470); in 1 of these syphilis was probably acquired. The duration of false positive reactions was twenty-four days to fourteen weeks. Of the 86 infants with false positive reactions, the reactions of 36 per cent had become negative in four weeks; of 74 per cent, in eight weeks; of 93 per cent, in twelve weeks, and of 97.7 per cent, in fourteen weeks. Correlation of the number of false positive serologic reactions with the time of mother's treatment shows a higher percentage of such reactions in the infants of mothers treated in the last trimester of pregnancy. In a comparison with statistics based on offspring of mothers treated with arsenicals and bismuth it was found that a greater number of false positive serologic reactions can be expected for the infants of mothers treated with penicillin.

In a review of infectious mononucleosis Dalton [85] discusses the false positive serologic reaction for syphilis that sometimes occurs as a confusing feature of this disease. Positive Wassermann and Eagle

84. Moon-Adams, D.: False Serologic Tests for Syphilis in Infants of Treated Mothers, New York State J. Med. **48**:1819-1821 (Aug. 15) 1948.

85. Dalton, W. W.: Infectious Mononucleosis: A Review of the Disease, J. Indiana M. A. **41**:1021-1025 (Oct.) 1948.

flocculation reactions for syphilis have been reported for some patients with infectious mononucleosis (approximately 191 between 1930 and 1942). The false positive reactions returned to normal within nine weeks in nearly all the reported cases. The author recommends that blood tests be repeated over a twelve week period before antisyphilitic treatment is instituted, when it is reasonably certain that the reactions are falsely positive.

Hemolytic anemia in a 50 year old white man was reported by Rubenstein [86] because a transient, strongly positive Wassermann reaction was found with negative reactions in the Kahn and Kline tests. The Wassermann reaction declined gradually with the receding hemolytic jaundice and after twenty days' observation was negative.

Perrot [87] reports the results of testing the serums of 390 persons thirteen and thirty-one days following vaccination against smallpox. At thirteen days, 55 serums gave some degree of positive reaction with one or more tests; 44 serums gave a single positive or doubtful reaction, and 11 gave several. At thirty-one days all serums were negative to all tests except the Meinicke, which gave 1 doubtful, 2 positive and 1 strongly positive reaction. These results suggest that vaccination is an appreciable factor in the causation of false positive reactions for syphilis but that such false reactions are weak and of short duration.

A careful analysis of the positive serologic reactions by Rosenthal and Widelock [88] before and after the mass program of vaccination against smallpox carried out by the New York City Department of Health in April 1947 failed to show any increase in the number of positive serologic reactions. The effect of vaccination against smallpox on the serologic tests for syphilis was studied on 133 vaccinated persons. Of this group 9 per cent acquired a positive reaction to one or more of the four tests employed (Mazzini, Kahn, Kolmer and VDRL slide [cardiolipin-lecithin antigen] tests). In most cases two and often three of the four tests gave negative reactions during the observation period of eight weeks. Most of the positive reactions were associated with an accelerated vaccinia.

Of 5,000,000 persons vaccinated against smallpox in New York city during April and May 1947, a random sampling was made of approximately 25,000 who returned for a reading of the vaccination site.

86. Rubenstein, M. A.: Transient Positive Wassermann Test for Syphilis in Acute Hemolytic Anemia, J. Lab. & Clin. Med. **33**:753-756 (June) 1948.

87. Perrot, H.: Portée pratique des réactions sérologiques syphilitiques positives apparaissant après vaccination jennerienne [réactions dites "faussement positives biologiques"], Bull. et mém. Soc. méd. d'hôp. de Paris **64**:124-128 (Feb. 6-13) 1948.

88. Rosenthal, T., and Widelock, D.: False Positive Serologic Tests for Syphilis Following Smallpox Vaccination, Am. J. Syph., Gonor. & Ven. Dis. **32**: 365-369 (July) 1948.

Greenberg [89] presents a report of the results in these 25,000 persons. There occurred 9 per cent of false positive serologic reactions for syphilis in a group of 133 known nonsyphilitic subjects, but these did not offer difficulties in distinguishing them from true reactions.

Tompkins [90] was also fortunate in being able to study serologically a fairly large group of patients after mass vaccination in 1947 in New York state. The material for study consisted of two series of successfully vaccinated persons, 23 and 54 nonsyphilitic persons respectively. The author concludes after the experience in New York city that the percentage of reacting serums during the period in which a large number of residents were vaccinated was not larger than in the period preceding or following it. Results of tests show no influence attributable to vaccination. False reactions in serologic tests for syphilis following vaccination for smallpox are due to reagents used and to technics of testing. Reactions are commoner with lipid antigens than with cardiolipin antigens. These are also more liable to occur in precipitation tests than in complement fixation tests. In the New York state quantitative complement fixation tests with cardiolipin antigen reactions are rare when the temperature of fixation is 3 to 6 C.

In another study de Lavergne and co-workers [91] subjected the serums of 9,842 Italian workmen, who were employed in French plants and lived in a lodging center, to the Wassermann test. The reactions of 144 serum specimens (1.4 per cent) were found to be positive. The men were vaccinated against smallpox, and the Wassermann test was repeated within twenty-eight days after the vaccination. Of 108 serum specimens with negative reactions before the vaccination, 19 (17 per cent) showed positive reactions, with 14 weakly positive and 5 strongly positive. Of the 14 weakly positive which had become positive as a result of the jennerian vaccination, 11 turned negative spontaneously within one hundred and fifteen days, 1 turned negative in one hundred and seventeen days, 1 in one hundred and twenty-nine days and 1 in one hundred and sixty-five days after the vaccination.

Universal Serologic Reaction with Lipid Antigen.—Kahn and his co-workers have presented a series of studies dealing with the technical procedure and significance of the universal serologic reaction.

Kahn [92] developed the universal serologic technic in 1946, and it has been studied in human beings in health and certain diseases (syphilis,

89. Greenberg, M.: Complications of Vaccination Against Smallpox (Syphilis), Am. J. Dis. Child. **76**:492-502 (Nov.) 1948.

90. Tompkins, V. M.: Nonspecific Reactions in Serologic Tests for Syphilis—Relationship of Vaccinia, New York State J. Med. **49**:1189-1190 (May 15) 1949.

91. de Lavergne, V.; Watrin, J., and Cauchois, J.: Vaccination jennérienne et réaction de Wassermann, Progr. méd. Paris, **77**:123 (March 24) 1949.

92. Kahn, R. L.: Universal Serologic Reaction with Lipid Antigen, Am. J. Clin. Path. **19**:347-360 (April-May) 1949.

tuberculosis, malaria and leprosy) and in some animals under normal and under experimental conditions. The technic in the universal serologic test follows the same principle as the Kahn precipitation test. The antigen employed is an alcoholic extract of heart muscle as used in tests for syphilis. The test employed in the universal reaction is based on a precipitation technic which combines different serum dilutions, sodium chloride concentration and incubations at cold temperatures as variables. These three variables form the basis of the different serologic patterns noted under conditions of health and of disease. Nine sodium chloride solutions are prepared: 0, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1 per cent respectively. Serial serum dilutions are prepared with each of the nine saline solutions in the ratio of 1:1, 1:2.5, 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. The Kahn antigen is prepared with 0.9 per cent saline solution and the ratio of serum antigen employed. Zone I refers to serum-antigen mixtures with saline solution of 0 to 0.3 per cent. Zone II are setups containing 0.6 to 1.2 per cent saline solution. Zone III are setups containing 1.5 to 2.1 per cent saline solution. Tests were read before incubation and after four and twenty-four hours of incubation in the refrigerator. Some two hundred serums of normal human beings were tested. In normal serums not incubated there was no precipitation. After four hours of incubation in the refrigerator precipitation occurred in zones I and III, whereas after incubation for twenty-fours hours precipitation is more marked in zones I and III. The clinical value of this universal serologic reaction is still to be established. It has been found that these serologic patterns undergo changes in patients with certain diseases, e. g., syphilis and tuberculosis. An investigation of these changes and determination of the normal patterns serve as controls. It is postulated that the nature of the universal serologic reaction in human beings may represent normal wear and tear of tissue in which lipids are liberated which act as antigens to stimulate the formation of specific antibodies and these react with tissue lipids in vitro. If this reaction represents a reaction of natural antibodies to lipids and these antibodies are stimulated by lipids liberated in normal wear and tear, then this reaction may prove valuable as a serologic indicator of normal wear and tear.

Kahn and Baribeau [93] present serologic patterns of universal reactions with lipid antigen obtained from serums from different animals. Serums studied were from cows, pigs, rabbits, horses, dogs, guinea pigs and mice. Cow, pig and rabbit serums were found to give similar serologic patterns. Marked precipitation was observed before and after incubation in zones I and III. Some precipitation occurs in zone II with stronger concentrations of serums. Horse, dog, guinea pig and mouse serum each

93. Kahn, R. L., and Baribeau, B. J.: Universal Serologic Reaction with Lipid Antigen: II. In Animals, Am. J. Clin. Path. **19**:361-368 (March) 1949.

gives a distinct serologic pattern, though the reactions are less marked. Although a variety of serologic patterns is noted, a common characteristic is that precipitation is intensified by incubation at cold temperatures.

Kahn and McDermott[94] examined 300 syphilitic serums by the universal serologic technic. In syphilitic serum the outstanding characteristic is the occurrence of precipitation without incubation of the serum-antigen mixtures. After incubation at cold temperatures, Kahn and McDermott noted, there was an increase in precipitation in zones I and III but little in zone II. The precipitation is also more marked in syphilitic serum. The disease does not call forth a new reaction but produces an enhancement of a previous existing reaction. In syphilis the serologic pattern may represent a result of destructive processes associated with syphilitic infection. Two situations may occur that affect the lipid antigen-antibody reactions: 1. Some increase over the normal liberation of tissue lipids occurs which results in a corresponding increase in antibody production. 2. A marked liberation of particular lipids occurs, and these call forth the production of antibodies characteristic of syphilis. It is not likely that the lipids liberated in syphilitic infection are totally different from those liberated in health, for the same lipid antigen is employed in vitro in the detection of syphilitic and normal antibodies to lipids. The universal serologic reaction in human syphilitic serum is characterized by a distinctive serologic pattern different from that given by normal human beings and by animals. Precipitation occurs rapidly when the serum is mixed with lipid antigen suspension and in most instances is enhanced by cold incubation, especially in the precipitation zone of approximately isotonic salt concentrations.

The universal serologic reaction in patients with lepromatous leprosy exhibits a distinctive serologic pattern. It differs from that in normal persons, in animals and in persons with syphilis. Serums of 55 patients with leprosy were tested with the universal serologic technic by Kahn, Baribeau and Vellaton.[95] Marked precipitation was noted immediately on mixing of the reagents. Precipitation was more marked with reduced saline concentrations (zones I and II), regardless of whether or not the specimens were incubated. After four hours of incubation practically all dilutions show precipitation. After twenty-four hours of incubation all serums show complete precipitation. In tuberculoid leprosy precipitation in zones II and III is absent without incubation, is less marked in zone II after four hours of incubation and is increased after twenty-four hours' incubation. Apparently the lipids liberated in syphilis and leprosy

94. Kahn, R. L., and McDermott, E. B.: Universal Serologic Reaction with Lipid Antigen: III. In Syphilis, Am. J. Clin. Path. **19**:401-407 (April) 1949.

95. Kahn, R. L.; Baribeau, B. J., and Vellaton, F. T.: Universal Serologic Reactions with Lipid Antigen: IV. In Lepromatous Leprosy, Am. J. Clin. Path. **19**:408-413 (April) 1949.

are increased in amount. In leprosy the increase may be associated with the degree of activity of the disease. The reaction may prove of value in differentiating lepromatous from other types of leprosy or serve as a serologic index of the response to therapy in the disease, relapses or spontaneous improvement.

The universal serologic reactions in human beings before and after malarial infection were also studied by Kahn.[96] The universal serologic reaction in malaria showed definitely intensified precipitation over the normal approximately one month after inoculation with Plasmodium vivax organisms, about twenty days after the beginning of the fever and about two weeks after its subsidence. This observation indicates that increased precipitation in the universal reaction in malaria develops after an incubation period.

By chance, Kahn[97] had been following the universal serologic reaction in a laboratory worker in whom reactivation of pulmonary tuberculosis subsequently developed. Shortly before roentgen ray diagnosis was made, there was an increase in precipitation, which increased still further with activation. Some 55 patients with advanced tuberculosis were also examined. Most of these patients were in the terminal stage. No increase in precipitation was noted in the universal reaction in far advanced miliary and terminal tuberculosis. The latter strengthens the belief that the universal reaction is a response requiring at least a minimal state of health, below which antibody may be depressed.

(To be continued)

96. Kahn, R. L.: Universal Serologic Reaction with Lipid Antigen: V. In Malaria, Am. J. Clin. Path. **19**:414-418 (April) 1949.

97. Kahn, R. L.: Universal Serologic Reaction with Lipid Antigen: VI. In Tuberculosis, Am. J. Clin. Path. **19**:419-423 (April) 1949.

News and Comment

GENERAL NEWS

First International Congress on Diseases of the Chest.—The First International Congress on Diseases of the Chest will be held at the Carlo Forlanini Institute, Rome, Italy, Sept. 17 to 20, 1950, under the auspices of the Council on International Affairs of the American College of Chest Physicians and the Carlo Forlanini Institute, with the patronage of the High Commissioner of Hygiene and Health, Italy, in collaboration with the National Institute of Health and the Italian Federation Against Tuberculosis. Further information may be secured from Dr. Chevalier L. Jackson, Chairman of the Council on International Affairs, American College of Chest Physicians, 500 North Dearborn Street, Chicago 10, Ill., U.S.A., or from Prof. A. Omodei Zorini, Carlo Forlanini Institute, Rome, Italy.

Book Reviews

Weitere Fortschritte in der Blutgerinnungslehre. By Karl Lenggenhager. Price, $5.50. Pp. 243, with 64 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1949.

This is a monograph on the author's researches in blood clotting. This work has not been well known in the United States, and the book gives the opportunity to study and to appreciate the author's contributions to an important chapter of medicine. His experiments refute the belief that the blood platelets initiate the clotting by releasing thromboplastin. He shows that the plasma contains a prothromboplastin (he names it prothrombokinin) which becomes thromboplastin by the action of a substance discovered by the author, named thrombokatalysin. This occurs in low concentration in the circulating blood and may be identical with a fraction of trypsin. The blood of hemophiliac persons is deficient in thrombokatalysin, and the addition of this substance to hemophilic blood makes it clot in normal time. The last part of the monograph deals with experiments on the anticoagulants hirudin, heparin and apicur (bee venom).

Synopsis of Medicine. Ninth edition. By Henry L. Tidy. Price, $7.50. Pp. 1,243. Williams & Wilkins Company, Mount Royal & Guilford Aves., Baltimore 2, 1949.

The new ninth edition of this well known book has grown to no less than 1,243 pages, but it is still a handy 12mo. It is indeed a synopsis, with a vast amount of material set down in one-two-three order, and should be invaluable for quick reference and orientation. Since there is no discussion, however, some of the unqualified statements and directions for therapy may actually be dangerous in the hands of those not already well qualified.

Minutes of the Seventh Streptomycin Conference. Prepared and edited by the Veterans Administration Area Office, Washington, D. C., 1949. Pp. 360.

This symposium presents data contributed by fifty-four hospitals participating in continued investigations on the applications and indications for the use of

streptomycn. The Veterans Administration, the Army, the Navy, the United States Public Health Service, the Army Institute of Pathology, the National Research Council, the Food and Drug Administration, the American Trudeau Society and the National Tuberculosis Association are represented.

An excellent summary is presented, dealing with the development of resistance by the tubercle bacilli to various regimens of treatment with streptomycin. The toxicity and comparative effectiveness of streptomycin and dihydrostreptomycin are critically analyzed, and numerous protocols are included. It is concluded that considerable caution should be used in the administration of this antibiotic, and that disturbance of the eighth nerve, renal dysfunction, cutaneous manifestations and depression of bone marrow must be watched for.

The use of streptomycin in pulmonary, genitourinary, meningeal, miliary and extrapulmonary tuberculosis, as well as in various surgical prophylactic procedures, is dealt with in detail. All subject matter is clarified by the generous use of charts and tables.

The volume is concluded with protocols for the treatment of tuberculosis, as revised after the Seventh Streptomycin Conference.

Leitfaden der Blutmorphologie. By L. Schudel. Price, $3. Pp. 47, with 14 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1947.

This volume is a veritable dictionary of blood cells, including those all-important variants which arise in pathologic conditions. It is a nicely organized work concerning the pictures found in blood dyscrasias. Though it deals entirely with the peripheral blood and does not include clinical manifestations or bone marrow pictures, it is a most adequate study, complete with proper smearing and staining technics.

It is unfortunate that this bulletin is paper bound and thus not prepared for the hard use it may encounter.

Digitalis and Other Cardiotonic Drugs. By Eli Rodin Movitt. Second edition. Price, $5.75. Pp. 245, with 18 illustrations. Oxford University Press, Amen House, Warwick Sq., London, E.C.4, and 114 Fifth Ave., New York 11, 1949.

This brief and concise monograph is a revised edition of the original, which appeared in 1946. This volume is larger by 53 pages. Additional material pertaining to the newer concepts of cardiac failure is added. The book also contains seven new charts and a slightly larger bibliography. Other than for these additions it is unchanged.

Dr. Movitt discusses in detail the various preparations of digitalis now available. He stresses the superiority of the purified glycosides over the preparation of the whole leaf. He appears to be an enthusiastic adherent to the single dose technic of digitalization and modestly warns of the dangerous consequences resulting from toxic doses of these drugs.

The volume nicely summarizes the historical and pharmacologic action of the cardiotonic drugs. It is well illustrated with charts and tables.

Einfuhrung in die innere Medizin. By Hans Julius Wolf. Price, $7.25. Pp. 608, with 69 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1948.

This is a short textbook of internal medicine for juniors. Each chapter begins with the pathophysiologic basis and the important examination methods of the particular group of diseases. This arrangement facilitates the understanding and

stimulates the cooperation of the student. Very instructive is the introduction to electrocardiography. Unfortunately, the author has overlooked in many instances the progress of internal medicine during the last ten years. He does not mention the newer tests of the hepatic functions, the treatment of pneumonia and epidemic meningitis with penicillin, the treatment of exophthalmic goiter with thiouracil and related drugs, the surgical treatment of bronchial carcinoma or other valuable achievements of medicine.

Epilepsy and Convulsive Disorders in Children. By Edward M. Bridge, M.D. Price, $8.50. Pp. 670, with 36 illustrations. McGraw-Hill Book Company, 330 42d St., New York 18, and Aldwych House, Aldwych, London, W.C.2; 1949.

Results of more than sixteen years of experience with epilepsy, seizures and convulsive disorders in children, combined and correlated with a definitive review of the literature of the same and related subjects has resulted in a book which could well serve as a guide for all who deal with epilepsy, particularly in children.

From the beginning, emphasis is placed primarily on the underlying forces which give rise to the symptoms: hereditary, physiologic and environmental factors and personality. At the same time there is adequate discussion of diagnosis and of manifestations of the diseases, along with details of management and treatment.

Of utmost importance is the practical treatment of the psychiatric and sociologic aspects of the diseases as they affect the patient, the parent and the community.

This book is recommended to pediatricians, neuropsychiatrists, parents and social workers: in short, to all who are responsible for management of the child afflicted with seizures.

Fundamentals of Otolaryngology. By Lawrence R. Boies. Price, $6.50. Pp. 443, with 184 illustrations. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1949.

This small manual of the commoner disorders of the ear, nose and throat has been prepared primarily for the undergraduate student. Dr. Boies and his associates have written the text in a somewhat brief but concise and factual manner. These qualities would be an asset for undergraduate students with limited time allotted to otolaryngology.

The chapter on Physiology of the Nose and the one on Headache and Neuralgia are exceptionally well presented. The section on the nose is somewhat more thorough than that on the ear. Inflammatory diseases of the labyrinth are not described, perhaps because of their rarity. However, in the reviewer's opinion two or three pages allotted to the fundamental features of this type of infections of the temporal bone would have made a better rounded section on the ear.

Within the limitations of the purpose of the book and the space available the authors have prepared a good, concise text, with well chosen illustrations, well adapted for medical students or for use as a quick reference for the general physician.

Diseases of Children. By Donald Paterson, M.D., and Alan Moncrieff, M.D. Price, $10. Pp. 1033, with 380 illustrations. Williams and Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2.

As a source of English and European methods in pediatrics, this book probably is a good representative text. However, it is evident that much of the material is based on American publications. As is true of all such texts, it tries to cover

the entire field of pediatrics and is necessarily sketchy in its discussions of many conditions. It seems to the reviewer not to offer more than the good standard texts of pediatrics published in the United States, though it is well written and easily read.

As in any text which is published during a period of rapidly increasing knowledge of useful antibiotics and chemotherapeutic agents, treatment is its weak point. Because of this, its outlook is not as bright as it could be at the present. One is struck with the futility of putting much time into writing a text which will be behind the times so soon.

Textbook of Neuropathology. By Ben W. Lichtenstein. Pp. 474, with 279 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1949.

The author is well qualified to write such a textbook as this. The fundamentals of neuropathology are adequately covered. The material is well organized and concisely presented with unusual clarity. The clinical and anatomic manifestations of various diseases and their clinicopathologic correlation are recorded so as to be easily understood.

A clinical supplement in which the numerous neurologic syndromes are briefly defined should be of considerable value to the medical student and the practicing physician. The technical supplement should prove useful to those engaged in preparing microscopic sections. The illustrations are in general good and well selected. The table of contents and complete subject index add greatly to the value of the book for reference.

This volume is intended especially for medical students, for whom it should ultimately be recognized as an excellent textbook. It should also be valuable and popular among pathologists and practicing physicians.

Geriatric Medicine. By E. J. Stieglitz. Second edition. Pp. 773, with 180 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5: 7 Grape St., Shaftesbury Ave., London, W.C.2.

The second edition of this volume follows the same plan as the first, which is, as pointed out in the preface, "The application of understanding of the changes which come with senescence to all the facets of medical practice." The forty-seven contributing authors, all qualified in their fields, seem to have accomplished this goal.

The first section of the book deals with the anatomic, physiologic, psychiatric and sociologic aspects of aging. Additional chapters are devoted to medical care of the normal aged person and to surgical management, anesthesia and physical therapy in senescence.

Following this, the diseases prevalent in or peculiar to the declining years are discussed under section headings, such as Disorders of Metabolism, Disorders of the Respiratory System, Disorders of the Skeletal System and Disorders of the Cutaneous System. Etiology, diagnosis and treatment, on the whole, are covered adequately enough for the practitioner to refer to the volume with confidence.

There is little included in this book that cannot be found in standard texts, but it serves a useful purpose in mentioning only that which is pertinent to old age. The chapters on medical care of the normal aged, mental changes in the elderly and nutritional problems seem most valuable. "Geriatric Medicine" may be recommended to all physicians who are confronted with problems in elderly patients.

Obesity. By Edward H. Rynearson, M.D., F.A.C.P.; from the Division of Medicine, Mayo Clinic; Rochester, Minn.; Associate Professor of Medicine, Mayo Foundation, and Clifford F. Gastineau, M.D., Fellow in Medicine, Mayo Clinic. Cloth. Price, $3.50. Pp. 134, with 18 figures and illustrations. Charles C Thomas, Publisher, 301-327 E. Laurence Ave., Springfield, Ill.

In dealing with the subject of obesity, the authors of this book say, in essence, "don't eat so much!" They point out the various causes that are advanced to explain overweight and then show that, with exceedingly rare exceptions, all forms of overweight respond in a characteristic manner to low calory diets. The very few contraindications to weight reduction are considered. Reduction diets and the method of calculating them are discussed adequately. Accessory methods, such as the use of drugs that elevate the metabolism and drugs that discourage the appetite, are mentioned, usually to be condemned. This is a valuable practical handbook to guide the physician in dealing with his overweight patients.

Observations of the Pathology of Hydrocephalus. By Dorothy S. Russell. Price, 6 shillings. Pp. 138, with 90 illustrations. His Majesty's Stationery Office, York House, Kingsway, London, W.C.2, 1949.

This well illustrated, brief monograph is, according to this reviewer, the best treatment to date of the subject of hydrocephalus, with excellent discussions of the pathogenesis of this condition.

The author points out that hydrocephalus is always due to a single cause, that of obstruction. Such obstruction may be caused by a congenitally malformed aqueduct, by atresia following inflammatory conditions, by periaqueductal gliosis, by variations in the Arnold-Chiari malformation or by tumors. The author feels that the term "idiopathic" is not justified in hydrocephalus.

Of course, obstruction in the absorptive mechanism, the subarachnoid villi, may be one of the causes of hydrocephalus. It is truly a form of obstruction.

Advances in Internal Medicine. Volume 3. Edited by William Dock, M.D., of the Long Island College of Medicine, Brooklyn, N. Y., and I. Snapper, of the Mount Sinai Hospital, New York. Cloth. Price, $8.50. Pp. 478, with figures and illustrations. Interscience Publishers, Inc., 215 4th Ave., New York 3.

This is the third volume of a series dealing with advances in internal medicine. A fourth is in preparation. This volume is, in reality, a series of nine monographs, for the most part on totally unrelated subjects and each by a different author or authors. It is a bit difficult to see how the selection of material was made, but whatever the method of selection the subjects are dealt with in an excellent fashion. To review this book adequately would require a review of each of the monographs. It must suffice to say that the following rather specialized subjects are dealt with in a highly competent manner: Use of British Anti-Lewisite; Current Concepts of Hemolytic Anemia; Host, Drug and Parasite Factors that Modify the Therapeutic Action of Penicillin; Streptomycin in the Treatment of Tuberculosis; Histoplasmosis; Treatment of Hyperthryroidism with Anti-Thyroid Compounds; Diagnosis of Disease by Enzymic Methods; Plasma Fractionation; Mechanism of Acclimatization to Heat, and Modern Therapeutic Agents Used in Neurologic Conditions.

In dealing with such rapidly changing subjects as some of these, for instance streptomycin or penicillin, some advances are sure to take place after the work

has been completed. That has happened here. It is interesting to note the experiments with adrenocorticotropic hormone (ACTH) in connection with acclimatization to heat. Since then this hormone has become famous for quite another reason.

It is safe to say that any one who wishes any information concerning the subjects listed will find it here or will find a bibliography that will give him a thoroughgoing acquaintance with the subject.

The Common Form of Joint Dysfunction: Its Incidence and Treatment. By William Kaufman, M.D. Price, $8.75. Pp. 208, with 35 illustrations. E. L. Hildreth & Co., Brattleboro, Vt.

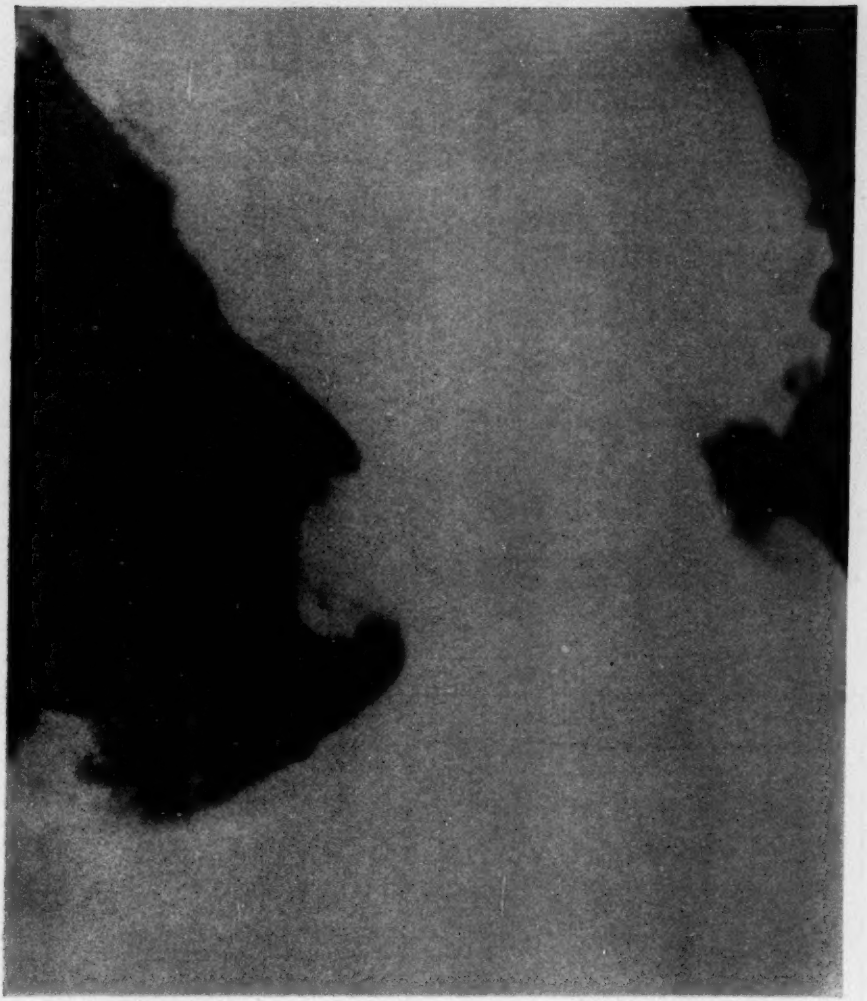
This monograph proposes the thesis that inadequacy of niacinamide ("aniacinamidosis") is the commonest cause of articular dysfunction. This disorder is reputedly "exceedingly prevalent in many individuals [with or] without joint complaints or clinically obvious arthritis" (page 3). Also "exceedingly prevalent in the group of patients studied" were psychosomatic symptoms and signs (page 130).

Data are based on a study of 455 patients aged 4 to 78 years, seen over a two year period from 1945 to 1947 inclusive. A weighted average of twenty joint measurements constituting a "joint range index" is used to classify severity, dosage requirement and response to treatment. The reported dosage of niacinamide ranged from 400 mg. to 4,000 mg. daily in divided oral doses. Additional components of the B complex, vitamin C and other medical and psychotherapeutic measures were also prescribed. "No matter what associated diseases the patient had, his joint dysfunction responded in a predictable way [correction or amelioration] to adequate therapy with niacinamide" (page 6). The paradox of normal joint range index with persistence of symptoms after treatment which was sometimes encountered was usually attributed to the presence of one or more of "four complicating syndromes frequently coexisting with joint dysfunction."

These are defined as (1) delayed post-traumatic articular syndrome (serious injury excluded), (2) chronic allergic syndrome, (3) sodium retention syndrome and (4) psychogenically induced, sustained hypertonia of somatic muscle. Differentiation of psychogenic or psychosomatic rheumatism from "the commonest form of articular dysfunction" is rather briefly and vaguely dismissed in a footnote (page 144). The author recognizes and regrets that he was unable to study repeated samples from the "untreated population"; likewise, the deficiencies which resulted from lack of controls, roentgenograms, photographs and pathologic studies. Sedimentation rates also were "not routinely determined," these data being obtained for only 172 of the 455 patients.

The author is to be complimented on the detailed description of some of his methods of study and treatment. Generalities frequently encountered in medical texts are thereby avoided. Excellent paper and printing make this book easy to read. A rather extensive bibliography is included, though references to the background of experimental studies relative to deficiencies of niacin are meagerly and tardily reviewed.

Numerous charts and graphs (42 pages) have been compiled, but, unfortunately, "no explanation can be given to account for the biodynamic mechanisms of niacinamide-induced improvements in persons with joint dysfunction" (page 191). The reader will readily agree with the author that much more information is needed before precise knowledge of the relation between niacinamide and dysfunction of joints can be fully evaluated.



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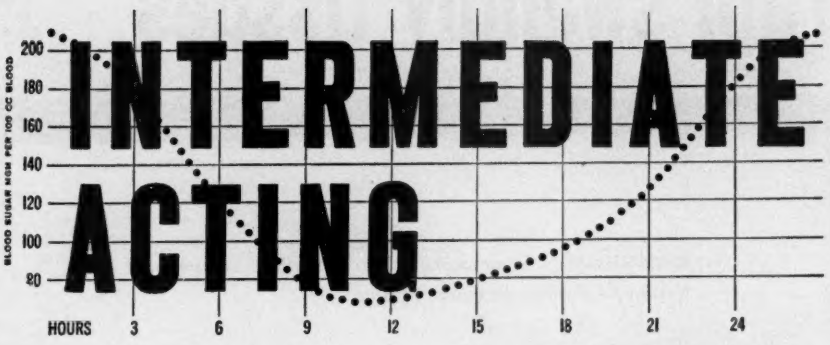

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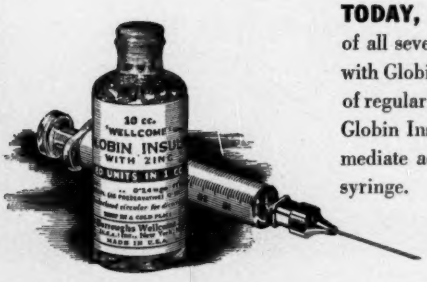

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1. Rohr, J.H., and Colwell, A.R.: Arch. Int. Med. 82:54, 1948.
2. Ibid Proc. Am. Diabetes Assn. 8:37, 1948.

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